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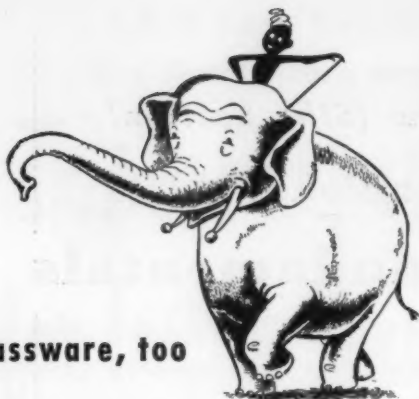
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CIBA Tissue Transplantation Symposium

A SYMPOSIUM on the Preservation of Normal Tissues for Transplantation was held in London, March 16-18, 1953, by The Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research. Though the majority of those participating in the symposium were British research workers in the fields of tissue transplantation and tissue preservation, guests from Holland, Switzerland, France, and the United States also attended. Among those from the United States who read papers or took part in the informal discussions which followed were: C. M. Pomerat, The Tissue Culture Laboratory, Medical Branch, University of Texas; E. J. Eichwald, Salt Lake General Hospital; Virginia J. Evans and W. R. Earle, Laboratory of Biology, National Institutes of Health, Bethesda; G. W. Hyatt, J. W. Pate, and W. R. Strong, U.S. Naval Medical Center, Bethesda; C. A. Hufnagel, Department of Experimental Surgery, Georgetown University; J. P. Merrill, Peter Bent Brigham Hospital, Boston; W. P. Longmire, Jr., U.S.A.F., Europe, and Department of Surgery, University of California; and B. O. Rogers, Department of Plastic Surgery, Presbyterian Hospital, New York City.

The program of the first day was opened by a lucid discussion of the general problems of immunity as they affect homotransplanted skin, by P. B. Medawar, of the Department of Zoology, University College, London. W. P. Longmire, Jr., reviewed the general surgical problems which arise in the clinical transplantation of homotransplanted tissues. An introduction to the techniques used in the cultivation of tissues over long periods of time on a large scale in tissue cultures by W. R. Earle, and a presentation of the recent findings in the transplantation of preserved nonviable tissue by J. W. Pate closed the first day's sessions.

The second day's program included the presentation of work performed by Ruth Deanealy, Audrey Smith, and A. S. Parkes (the symposium chairman), in the histology and storage of endocrine homografts at the National Institute for Medical Research, London. P. J. Gaillard, of the Institute for Experimental Histology, Leiden, Holland, gave a summary of his most recent findings in the

transplantation of cultivated parathyroid gland tissue in man. P. F. Jones, of the Department of Surgery, St. Bart's Hospital, London, reported the results of his experimental and clinical study in the use of foetal and neonatal endocrine homografts. The problems which confront those who are interested in the preservation of blood were reviewed by P. L. Mollison, of the Postgraduate Medical School, London. J. E. Lovelock, National Institute for Medical Research, London, explained the biophysical changes which occur when living cells are frozen. Methods for analyzing thermal stress with tissue culture techniques were demonstrated by a noteworthy film on the reaction of living cells to thermal stress by C. M. Pomerat.

The third and final day's program included a discussion by R. E. Billingham, Department of Zoology, University College, London, on the storage of skin, with emphasis placed upon the use of glycerol in storage at reduced temperatures. F. K. Sanders, Department of Zoology, Oxford, spoke on the preservation and application of nerve autografts and homografts. The clinical use of preserved arteries and other tissues was described by C. G. Rob and H. H. G. Eastcott, Department of Surgery, St. Mary's Hospital, London. Many new experimental and clinical observations on the transplantation of blood vessel homografts and heterografts were reported by C. A. Hufnagel. A thorough review of the current status of corneal transplantation and the methods of preserving cornea was presented by B. W. Rycroft, Corneoplastic Unit, Queen Victoria Hospital, East Grinstead. The last paper of the symposium was given by W. R. Strong, who fully detailed the methods of operation and management of a tissue bank, using as his guide the bank established by the U.S. Navy at Bethesda.

The symposium was limited to about 45 participants, making it a closely knit, smoothly functioning, working conference. Much of the credit for the remarkable efficiency with which the symposium moved along can be attributed to the efforts of Dr. Gordon E. W. Wolstenholme, director of The Ciba Foundation, and his staff. A book is to be published containing the proceedings of the symposium. It will include all the papers and the complete transcribed discussions which followed them.

BLAIR OAKLEY ROGERS

Department of Plastic Surgery,
Presbyterian Hospital, New York City

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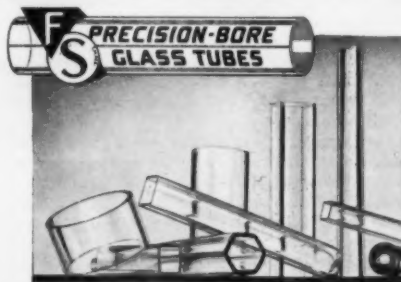
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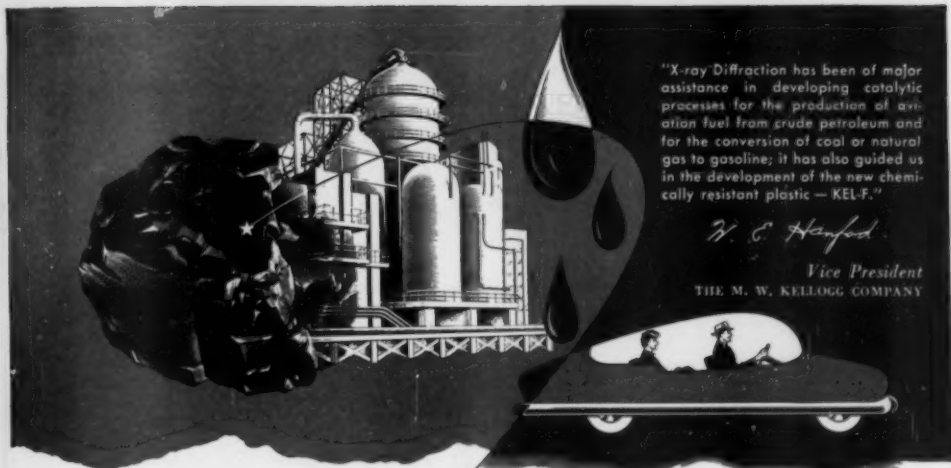
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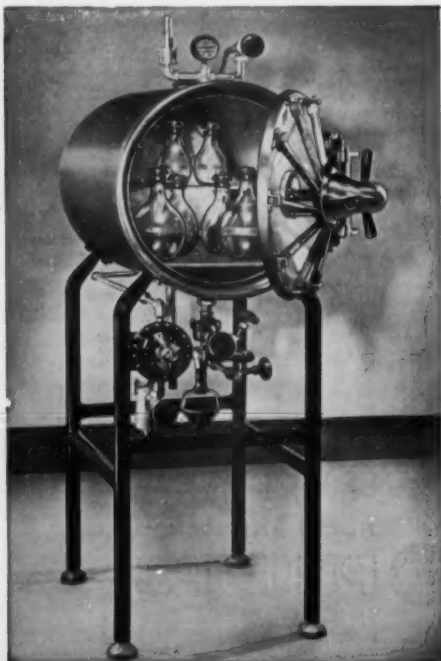
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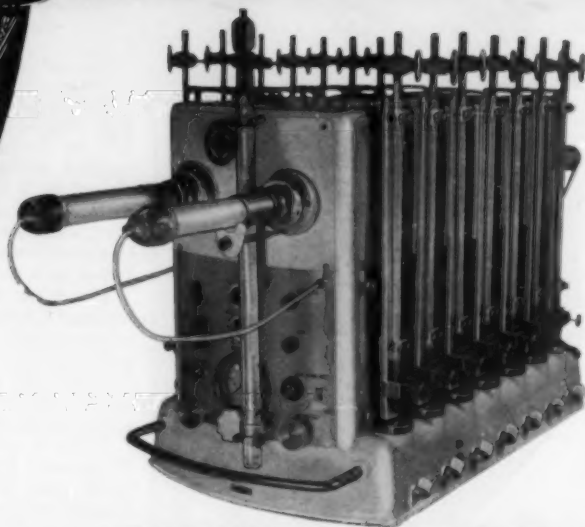
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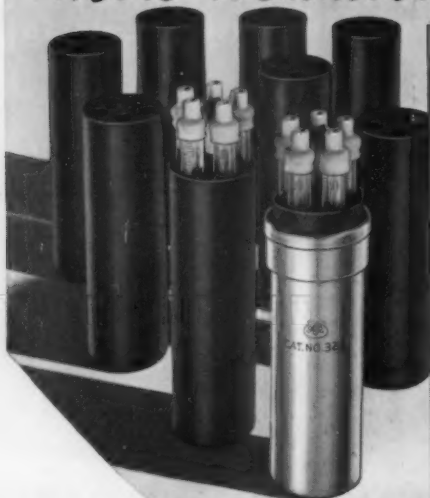
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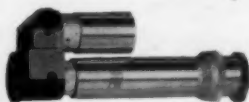
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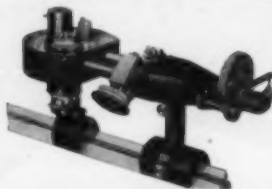
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On the Nuclear Envelope¹

Norman G. Anderson²

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

THE VAST SPECTRUM of genetically controlled cellular differentiations depends ultimately, according to our present concepts, upon the interchange of chemical substances between the genes and the cytoplasm. No ultra-long-range physical forces which might exert controlling influences through the chromosomal sheath or the nuclear envelope have been adequately demonstrated (1). In any discussion of nuclear function it therefore becomes necessary to consider the factors limiting the rate of movement and the nature of material diffusing between the substance of the chromosomes and the rest of the cell. Of central importance is the permeability of the nuclear envelope (karyotheca), which will be considered in the present paper.

It has been suggested that the nucleus possesses a semipermeable membrane and may exhibit osmotic properties (2, 3). If this is correct and if the membrane consists of the peripheral segments of chromosomal sheaths (4) possessing the same properties, then the genetic substance would appear to be permanently imprisoned behind a semipermeable barrier admitting only such substances as simple salts (3, 5), nucleotides (3), and certain dyes (6). Even without the experimental evidence to the contrary to be cited, such a conclusion is difficult to accept on purely theoretical grounds, since it does not appear possible that the complexities of genetic expression could be mediated entirely through simple salts and substances of comparatively low molecular weight (<1000). It should be pointed out that the nuclear volume changes which previously had been considered to be evidence for osmotic behavior have been shown, in the case of the isolated rat liver nucleus, to be due to the effects of electrolytes on a predominantly anionic gel (7). Detailed studies have given no evidence of semipermeability.

The assumption that high-molecular-weight substances (gene products—presumably enzymes or nucleoproteins) may move from the site of their formation to other points within the nucleus to form a component of the nuclear sap or of the nucleoli, but may not diffuse across the karyotheca, only partially alleviates the difficulties, since only three mechanisms are then apparent for moving these substances to the cytoplasm across the nuclear membrane. The first of these, vacuolar or nucleolar extrusion through temporary openings in the nuclear envelope (8), may occur in special instances but has not received suffi-

ciently wide demonstration to be considered as generally occurring. Except in the case of frog oocytes, the evidence has been obtained, for the most part, from the study of fixed and stained preparations, which are often difficult to interpret. The second mechanism, the release of gene products from the nucleus after the breakdown of the nuclear envelope during mitosis, deserves serious consideration.

If genetically important substances are received by the cytoplasm only during cell division, it is evident that the differentiated, nondividing cell lacks continuous nuclear direction. A number of observations suggests that this is not the case, chief of which is the well-known fact that most cellular differentiation occurs in cells which do not divide. Evidence for a high rate of nucleic acid synthesis in the nucleus of such a highly differentiated cell as the mammalian neuron has been found (9). Studies on the effects of enucleation in protozoa (10) and on regeneration in a variety of cells, especially *Stentor caeruleus* (11), provide very direct indication of active nuclear participation in complex synthetic activity. Here all available evidence shows that the nucleus alone is capable of directing the reconstruction of a complex three-dimensional structure complete with an integrated system of organelles. Further, the nuclear desoxyribonucleic acid (DNA) must be in a highly polymerized form to organize this process successfully (12). The most conclusive demonstration that the breakdown of the nuclear envelope is not a prerequisite for the release of gene products is based upon the well-known finding that the nuclear envelope in ciliates never breaks down but continues intact from one division to another. Similar envelope persistence during mitosis has been described in the *Tradescantia* stamen-hair cell (13). A number of other important evidences for nuclear-cytoplasmic interactions during interphase based on cytological studies have been carefully reviewed by Stern (14) and by Huskins and Steinitz (14a).

The third possible mechanism for the movement of macromolecular gene products to the cytoplasm in the presence of a semipermeable membrane involves the formation of the membrane from the gene products themselves. These would be presumed to accumulate at the nuclear-cytoplasmic interface, and to dissolve gradually on the cytoplasmic side. Certain regulatory properties are inherent in such a system. Excess nuclear synthesis would result in the formation of a thick membrane, tending to slow down the diffusion of low-molecular-weight substrate compounds into the nucleus until cytoplasmic solution of the envelope material again thinned the membrane. At any

¹Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

²It is a pleasure to acknowledge the helpful advice and criticisms of Carl P. Swanson.

one instant such an envelope could be impermeable to its own constituent macromolecules, yet still be serving as a mechanism for their transportation. The chief advantage of this theory is the simple explanation it provides for the breakdown of the envelope during cell division, to wit, cessation of nuclear synthetic activity coupled with continued cytoplasmic solution resulting automatically in envelope disappearance. While this theory deserves further experimental consideration, there is at present little evidence to support it. Studies on the stability of the nuclear envelopes of isolated nuclei and on isolated nuclear envelope preparations carried out in this laboratory suggest, on the contrary, that they are very stable structures permeable to macromolecules.

Evidence for complex nuclear-cytoplasmic interactions during interphase, occurrence of nuclear envelopes which persist during cell division, and difficulties encountered in attempting to explain the movements of gene products to the cytoplasm in the presence of a semipermeable membrane all support the generalization advanced here that genetically important (determining) substances move from the genes to the cytoplasm *through the nuclear envelope*. The nature and physical size of such substances then become the central problem. In the preceding discussion it has been assumed that these molecules have the general dimensions of soluble cytoplasmic proteins or nucleoproteins. While it is difficult to prove experimentally that nuclear control is *not* mediated through organic compounds of relatively low molecular weight (15) (<1000), numerous studies of the effects of thousands of synthetic substances and drugs on living cells have produced little evidence that any known compound is "genomimetic." For the present it can only be considered as most probable that the cytoplasmic expression of nuclear control (differentiation, regeneration, etc.), which involves the synthesis of an almost countless number of specific proteins throughout the estimated one million or so species comprising the plant and animal kingdoms, is mediated principally through the movement of macromolecules having the size characteristics of enzymes, structural proteins, and certain nucleoproteins.

NUCLEAR PERMEABILITY

Evidence from several sources indicates that substances of high molecular weight (>15,000) may pass through the nuclear envelope. Macromolecules possessing a higher anionic charge density than nucleic acids (heparin, sulfated polyanuronic acid, etc.) have been observed to enter isolated rat liver cell nuclei and to displace very highly polymerized DNA, which then passes rapidly out *through* the nuclear envelope without producing apparent changes in its structure (15a). Phosphatases are bound by isolated nuclei and are readily displaced by a number of proteins, including protamine (16). Recent work in this laboratory on the effects of nucleases on freshly isolated unfixed rat liver cell nuclei (17) has

shown that these enzymes penetrate such nuclei rapidly and produce characteristic effects in a surprisingly short time (ca. 30 seconds for ribonuclease and 1 to 4 minutes for desoxyribonuclease). Evidence for the penetration of isolated nuclei by bovine serum albumin, partially hydrolyzed gelatin (18) and hemoglobin has also been found. Nuclei isolated from a number of sources have been observed to lose protein rather easily (19). While these observations all support the view that the nuclear envelope is permeable to large macromolecules, it should be emphasized that results obtained with isolated nuclei must be interpreted with caution.

Evidence for the penetration of the nucleus by macromolecules in the intact cell comes from several independent sources. Antigens injected into whole animals have been subsequently demonstrated in tissue nuclei (20). The extensive work of Caspersson and his co-workers, indicating that nucleoproteins or nucleic acids are synthesized in the nucleolus and subsequently move to the cytoplasm (9, 21), has received considerable confirmation from studies involving the use of radioactive tracers (22). Jeener and Szafarz state that ribonucleic acid (RNA) moves from the nucleus to the cytoplasm in the form of macromolecules which do not sediment at 60,000 \times g, and are therefore smaller than the microsomes (23). Suggestive as these observations are, the unequivocal demonstration that *normal* macromolecular constituents of the cell readily cross the membrane in both directions is still lacking. Studies employing isotope techniques to demonstrate first the rapid synthesis of nucleolar RNA and second its subsequent movement to the cytoplasm assume that the RNA showing isotope incorporation is the same in both instances, and do not rule out entirely the possibility that the tag has actually moved across the nuclear-cytoplasmic interface as a component of a relatively small molecule.

Many of the instances cited as demonstrating the permeability of the nuclear envelope also demonstrate the ease with which large molecules may diffuse through the substance of the nucleus. The rapid effects of ribonuclease on the nucleolus, and the fact that dissolution of the interior by desoxyribonuclease takes place evenly throughout the body of the nucleus and not from the edge inward (17), suggest that the chromosomal material exists in the interphase mammalian somatic cell as an open gelwork. The almost free diffusion of proteins in similar gels is well known.

Recently it has been suggested that rat liver nuclei isolated in a sucrose- CaCl_2 mixture may have a protein-impermeable membrane, since a higher protein-DNA ratio was observed than is found, for instance, with citric acid (24). It was further suggested that loss of enzymes after disintegration with ultrasonic vibrations also demonstrated that the membrane was impermeable. It should be pointed out, however, that DNA has repeatedly been shown to combine with a wide variety of proteins while in a highly polymerized state (16, 25) but not when depolymerized. It would

be expected that the intranuclear DNA would hold a considerable amount of protein in rather loose combination. Since ultrasonic waves, which were used to destroy the nuclear envelope, depolymerize DNA with surprising rapidity (26), one would expect nuclear protein to be solubilized by this treatment. These experiments cannot be considered to bear directly on the protein permeability or impermeability of the nuclear envelope.

Numerous studies have been made of the distribution of substances between various isolated cell components and fractions (27). The interpretation of these is difficult and will be discussed at length elsewhere. Following the pioneering work of Donnee (28) on nuclear enzymes, Lang (29) demonstrated that glycolysis occurs in the nucleus. Glycolytic enzymes have also been demonstrated in nuclei prepared by the method of Behrens by Stern and Mirsky (30). Since the glycolytic system is characteristic of what we shall here term the *soluble continuous phase* (31), it would be expected to permeate the nucleus.

It should be emphasized that the existence of a permeable nuclear envelope does not imply uniform distribution of a great number of enzymes between nucleus and cytoplasm, since these may be expected to be adsorbed in varying degrees on particulate constituents.

THE STRUCTURE OF THE ENVELOPE

The substance of the nuclear envelope has been considered to be composed chiefly of basic proteins (32) with associated lipid (33), which, in fixed preparations, constitutes a "perinuclear layer" (34). Recent electron microscope studies on isolated envelope material from amphibian oocytes and amebae suggest the presence of at least two components or layers, one a continuous sheet, the other regularly perforate (35). Preliminary studies on mammalian somatic nuclei have not revealed the presence of similar perforate structures (36). It should be noted that all observations so far reported appear to have been made on air-dried material, the interpretation of which is equivocal, since resultant surface tension effects have been shown to cause extreme alterations in biological materials (37).

Observations on the effects of a number of solutions on nuclei isolated from rat liver and studied in the unfixed state have suggested that the envelope in this instance also is composed of two components, one a structural layer consisting either of a meshwork of fibrils or of a fine porous sheet (18). The extreme elasticity (7) favors the meshwork hypothesis. Associated with it is a second mobile component which is presumed to be rich in phospholipid. Normally, the mobile component is believed to be associated with the structural component in such a manner as to leave open the pores or interstices. Alterations in the composition of the medium (18) appear to cause the mobile component to move to the surface of the envelope and give rise to a continuous, protein-imper-

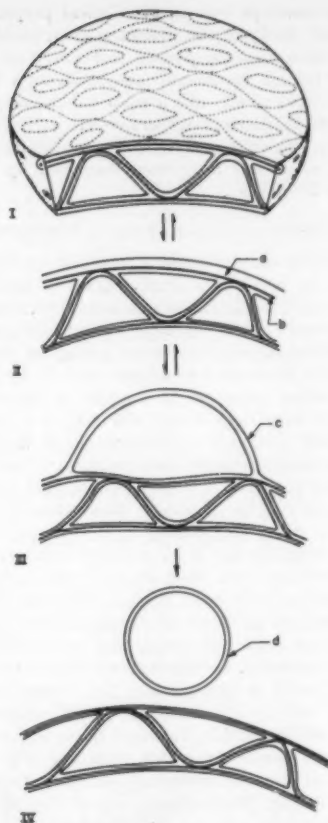


FIG. 1. Schematic diagram of nuclear envelope composed of a meshwork of fibrils coated with lipid material. Stage I shows portion of envelope as it appears to be in the freshly isolated nucleus. In stages II-IV the mobile lipid component (a) moves from the protein fibrils (b) to the surface to form a continuous sheet which may rise off to form blebs (c) or free floating spheres (d). Stages I-III are reversible.

meable sheet. This sheet may even lift off the nuclear surface to form either blebs or free-floating spheres. Bleb formation by the mobile component is easily reversible and may be demonstrated in isolated nuclear envelopes prepared by a combination of enzymatic digestion and treatment with nonionic media. A highly schematic diagram of the formation of blebs is shown in Fig. 1.

The two-component system proposed provides a mechanism for altering the permeability of the envelope, possibly in a cyclical manner. Histological studies often show the nuclear envelope to disappear unevenly during cell division, whereas observations on living grasshopper neuroblasts show it to disappear very suddenly and completely (38). Since efforts to fix the blebs formed on the surface of mammalian nuclei have not been successful (36), it is probable

that the envelope seen in histological preparations is composed chiefly of the structural component. During cell division this component is believed to dissolve, leaving behind the mobile component as a thin continuous sheet which, like a soap bubble, may lose its structure throughout its entire circumference very suddenly. Phospholipid vacuoles resembling the dissolving membrane have been described by Bungenberg de Jong (39).

ENVELOPE DISSOLUTION AT PROPHASE

Little experimental data is available on the mechanism of the breakdown or dissolution of the envelope during cell division. Lettré has considered that nucleic acids present in the envelope during interphase prevent proteolytic enzymes from acting on this structure (40). Movement of nucleic acid to the chromosomes during prophase is thought to leave the envelope proteins unprotected and subject to digestion. It has been proposed by Heilbrunn and Wilbur (41) and by Goldstein (42) that calcium, released from the cortex of *Nereis* or *Chaetopterus* eggs, activates a proteolytic enzyme of a type demonstrated by Gross (43). The ready digestibility of the envelope by proteolytic enzymes has been recently demonstrated in this laboratory (17). Such enzymes attack the DNA-nucleoprotein even more readily, however. The work of Monné and of Baud (44) suggests the possibility that surface-active substances may be involved in envelope breakdown. On the basis of studies on the stability of nuclei in homogenates, the author prefers to conclude that the structural component of the karyotheca is not digested in the usual sense, but becomes a part of the mitotic apparatus, returning to its former position at the nuclear-cytoplasmic interface at the end of division. The probable role of the envelope in mitosis has been stressed by Schrader (45).

ON THE FUNCTION OF THE ENVELOPE

The available information supports the view that the nuclear envelope is a porous structure, generally permeable to macromolecules, yet containing within itself a mechanism for markedly altering its own permeability. The point at which this system is poised may well vary during the life of the cell, but on the basis of the evidence presented, it is believed to be shifted toward high permeability in somatic cells generally. Germinal vesicles, which accumulate considerable material within their envelopes, may well be shifted to the other extreme. The exclusion of cytoplasmic particulates from contact with the chromatin may be the key to the peculiar structure of the envelope, since deoxyribonuclease, which readily attacks DNA in isolated nuclei, is generally bound to cytoplasmic particulates (46). Several authors, following Mazia's original work (46, 47) have inferred that this nuclease plays some role in DNA synthesis. It appears more probable, in the absence of any demonstrable synthetic activity by this enzyme, that it serves to destroy DNA which has become loosened

from an organized nuclear gel, and which cannot be allowed to survive in the cytoplasm. According to Painter (48), thousands of chromosomes are poured into the cytoplasm of developing oocytes by surrounding cells. This material becomes sufficiently depolymerized to be Feulgen-negative, but may well persist as polynucleotides. Schultz (49) has recently suggested that this phenomenon may account for the unexpectedly high DNA values obtained by gross analytical techniques (50). The necessity for mechanisms which will isolate and destroy products of defective synthesis or of accident at many levels of organization has been well pointed out by Crane (51). The nuclear envelope may well be part of such a system.

It is difficult to conceive of effective nuclear control of complex cytoplasmic processes, especially in cell regeneration, without the transfer of information (substances) from the cytoplasm back to the nucleus. Such a transfer would be analogous to the "feed-back" principle stressed by Wiener (52). The simplest controlling feed-back system applicable here is the one inherent in an equilibrium state where the product concentration controls the reaction rate. If the continuous soluble phase of the cell includes, as suggested here, the soluble proteins and nucleoproteins of the cytoplasm, then these substances may be considered to be in equilibrium with the genetic material itself. Alterations in the composition of the continuous soluble phase would then result in compensatory nuclear activity.

In conclusion, it must be said that information on the structure and function of nuclear components is still very meager. It is hoped that the results and concepts reviewed here will serve to stimulate greater interest in this difficult field.

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William Herbert Hobbs: 1864-1953

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WILLIAM HERBERT HOBBS was born on July 2, 1864, in Worcester, Mass., the son of Horace Hobbs and Mary Paine Parker Hobbs. The death of his mother the next year was followed within another year by the arrival of a stepmother. To his father and stepmother he owed much of his character, personality, and habits. At the age of 18 he had shot up from a small, anemic boy to his adult stature of 5 feet 10½ inches, with no more constitution and proportionally no more flesh. In spite of these handicaps he became a wiry, energetic, intensely vital man, who was able to round out a life of 88½ years, passing away in Ann Arbor, Mich., January 1, 1953.

Dr. Hobbs gave some attention to his lineage and wrote that his ancestors settled in the vicinity of Worcester, Mass., during 1620-1671. He traced every line he could find—155 in all—and learned that they

came from the British Isles and all bore British surnames, giving him a pure British-American ancestry, of which he was proud.

In a scholar's biographical sketch, life can be divided into academic periods. For Dr. Hobbs there were four: 25 years of study in America and Europe culminating, though not ending, with his doctorate at The Johns Hopkins University in 1888; 16 years at the University of Wisconsin as a teacher of mineralogy and petrology; 28 years building up a strong department of geology at the University of Michigan from a one-man department with 131 students to a ten-man department with 1035 students; and 18½ years after retirement. A full year for European study separated the two University appointments.

The story can also be told in terms of his major interests: drawing and mathematics from childhood to the year 1886-7, when his attention was transferred

to mineralogy and petrology under the influence of inspiring teachers; dynamic and structural geology from 1905 to the end of his fruitful life. There was an easy transition, as shown by his papers, from an interest in minerals and rocks to an interest in the two new fields.

For W. H. Hobbs life was not divided into compartments. Born and educated in glaciated areas of crystalline rocks or sedimentaries approaching crystallines, able to travel to the ends of the earth (both polar areas) and around its girth, tramping and studying in every continent, he developed a broad, interlocking concern about many phases of his field—glaciers, volcanoes, earthquakes, mountains, and faults. In pursuit of these subjects as problems, travel was essential. He followed several studies to the stage of publishing extended papers and fifteen books. His cosmopolitan mind and multiplicity of interests had room for politics, war, exploration, and conservation. To be more specific, he completed the course in designing patterns for industrial commodities in Worcester Free Institute of Industrial Science in his native town; attended The Johns Hopkins University as a graduate student from 1884 to 1888 and obtained M.A. and Ph.D. degrees at the same time. Then came a profitable year in Europe, where he was based at Heidelberg but collected and observed over Europe, up and down the Rhine, among Italian volcanoes, and in the Swiss mountains. Field work for the U.S. Geological Survey, mostly in western Massachusetts, more travel in Europe, and attendance at national and international geologic meetings added to Dr. Hobbs' knowledge of his subject and of men.

A Europe-African journey in 1905 and another in 1912 expanded the field of his experience. The polar controversies helped prepare him for his own polar research. In 1922 an exchange professorship with H. S. Brouwer, of Delft, Netherlands, aided him in the interpretation of his travel experiences to the Pacific coasts and islands in 1921 and 1923. The second of these trips included attendance at a Pan-Pacific Science Congress with all its valued connections, and both gave him much observational material for future reading and cogitation on problems of mountain arcs, volcanism, coral islands, and mineral deposits. In his later years he became keenly interested in, and wrote several papers about Trans-isthmian canals to connect Atlantic and Pacific.

Many speak of his intense vitality and infectious enthusiasm, which drew students into geology and fired many to pursue the rocky road for life. He was rarely too busy to advise students, to discuss their academic or personal problems, or to elaborate out of class points touched in the classroom. As a teacher he was inspiring, a tireless worker, and an unusually able administrator. His students retained a high esteem for him.

Although he sometimes appeared shy to acquaintances, Dr. Hobbs relished controversy and would pursue an argument fearlessly, with unflagging enthusiasm and zeal. Argument never seemed to dampen warm friendship or cordial high regard; and it ended with no continuing bitterness or hard feeling in either scientific or political fields.¹ He made friends with ease, and throughout his long life and extensive travels he accumulated a wide circle of correspondents among scientists and explorers and among political, naval, and military men. Through personal acquaintance he drew many famous men to Ann Arbor for addresses or conferences.

He had a keen sense of humor, as some of the incidents in his autobiography indicate, and could relish stories with the joke on himself. He loved the companionship with his fellow workers in whatever field he labored.

He was the recipient of honorary degrees, honors, and offices in many societies. Other explorers have seen fit to put his name on geographic features—five glaciers, three mountains, and other physical details of the earth, mostly in high latitudes. In the words of the last paragraph of a memorial prepared by three of his colleagues at the University of Michigan: "To his friends and colleagues at the University of Michigan the spirit of Dr. Hobbs was best typified by his springy, vigorous stride across the campus and his warm cheery greeting to one and all. His stride and his erect carriage remained unchanged throughout the years, as did his vigor and exuberant enthusiasm in all his scientific undertakings. . . . He was truly ageless and will always be a great inspiration to all who knew him."

¹ EDITOR'S NOTE: The editor can name a single exception to Dr. Hubbard's statement, but the fact that there was only one in so long a professional life provides eloquent support to the author's tribute and a commentary on the single adversary who failed to retain Dr. Hobbs' esteem.



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News and Notes

MIT Acoustics Laboratory

A Special Summer Program in Noise Reduction will be given in the Acoustics Laboratory at the Massachusetts Institute of Technology from Aug. 24 to Sept. 4, according to Ernest H. Huntress, director of the MIT Summer Session.

The program, designed to give engineers and scientists a working knowledge of means for noise reduction in industrial plants and in buildings, will be under the direction of Leo L. Beranek, associate professor of communications engineering and technical director of the MIT Acoustics Laboratory. He will be assisted by four members of the Acoustics Laboratory staff: Jordan J. Baruch, Richard H. Bolt, Robert B. Newman, and Walter A. Rosenblith.

Guest lecturers will include Kenneth N. Stevens and S. Labate of Bolt, Beranek and Newman, consultants in acoustics, and others from industry and building research.

"The problem of noise reduction in industrial plants and buildings has skyrocketed to importance because of recent court decisions favorable toward workers with partial loss of hearing," Professor Beranek points out. "Simultaneously," he continues, "architects have found their clientele demanding quieter living and working conditions."

The Special Summer Program at MIT will present methods of measuring a wide variety of noises, as well as techniques and materials for noise reduction. Lectures and demonstrations will cover the following topics: basic terminology and concepts, measurement of noise, psychological aspects, room acoustics theory, noise reduction in rooms, transmission of sound through structures, characteristics of noise sources, materials and structures for noise reduction, mufflers and vibration isolators, machinery and ventilation-system quieting.

Afternoons during the two-week period will be reserved for round table discussions and field trips. Facilities of the MIT Acoustics Laboratory will be used throughout the program.

Further information and application forms for the Special Summer Program in Noise Reduction may be obtained from the Director of the Summer Session, Room 3-107, Massachusetts Institute of Technology, Cambridge 39.

Scientists in the News

Herbert C. Brown, professor of chemistry, Purdue University, has been selected to deliver the 1953 Harrison Howe Lecture before the Rochester Section of the American Chemical Society. Dr. Brown, who was chosen for his contributions to stereochemistry and for his work in the synthesis of hitherto unknown boron hydride derivatives, will give the lecture Nov. 21.

Three new professors have been appointed at Yale University. Norman A. Cameron, professor of psychiatry and psychology at the University of Wisconsin, will be professor of psychiatry; Edward C. Hammond, director of statistical research for the American Cancer Society, will be professor of biometry; and Arnold D. Welch, professor of pharmacology at Western Reserve University, will be professor of pharmacology and chairman of the department.

Francis P. Dwyer, professor of chemistry at the University of Sydney, will be a visiting lecturer at Northwestern University during the next academic year. Dr. Dwyer is a specialist in the field of coordination compounds.

Walter Rudolphe Heumann has been appointed assistant professor of analytical chemistry at the University of Montreal. Dr. Heumann went to Canada from Europe in 1951, and has been concerned with the development of new products for Ayerst, McKenna & Harrison.

John A. Hipple has been appointed director of the Experiment Station, School of Mineral Industries, at the Pennsylvania State College. Dr. Hipple has been chief of the Atomic Physics Section of the National Bureau of Standards since 1947.

Shirley A. Johnson, Jr., has been named permanent director of the Denver Research Institute of the University of Denver. He has been associated with the university since 1947, and with the institute since 1948.

Georg Kahlson, of Lund University, Sweden, has received a grant from the Rockefeller Foundation to continue his research on the hormones, especially the hypophysis and the suprarenal glands.

Richard L. Kirk has been appointed director of production of the Atomic Energy Commission's New York operations office. Mr. Kirk has been associated with the national atomic energy program since 1944.

Alexander Koyre, of the Sorbonne, Paris, has been appointed Kemper K. Knapp visiting professor at the University of Wisconsin for the first semester of 1953-54. Dr. Koyre is a distinguished scholar in the field of history of science.

David Macht was presented with a medal and honorary membership in the Royal Academy of Pharmacy of Madrid. Dr. Macht was honored for his contributions in pharmacology and phyto-pharmacology.

Francis W. Maxstadt, associate professor of electrical engineering at the California Institute of Technology, has been appointed registrar of the institute.

Ernst Mayr, curator of the Whitney-Rothschild Collection of the American Museum of Natural History, New York, has been appointed professor of

zoology in the Museum of Comparative Zoology and Alexander Agassiz Professor of Zoology, Harvard University, effective July 1.

Lynn L. Merrill has been named professor of mathematics and head of the department at Stevens Institute of Technology. Dr. Merrill was formerly chairman of the Mathematics Department at Clarkson College of Technology, Potsdam, N. Y.

Charles B. Metz, associate professor of zoology at the University of North Carolina, has been appointed Frank R. Lillie Fellow in Embryology at Woods Hole, Mass., for the summer of 1953.

Eger V. Murphree, president of the Standard Oil Development Company, has received the Industrial Research Institute's 1953 medal, for leadership of industrial research contributing to the public welfare in war and peace.

Clarence N. Peiss, assistant professor of physiology at St. Louis University School of Medicine, has been named a John and Mary R. Markle Foundation Scholar in Medical Science for a five-year period, beginning in July of this year.

George Rieger, of the Hercules Powder Company's Sales Research Division, has been named program executive of the Plastics Section of the National Production Authority's Chemical Division. Mr. Rieger will be on loan to the government agency for six months.

Sir Robert Robinson of Oxford University, Nobel prize-winning chemist, has received the Priestley Medal of the American Chemical Society. Sir Robert was honored for researches on the chemicals involved in the life processes of animals and plants.

Harold P. Rusch, director of the McArdle Memorial Laboratory for cancer research at the University of Wisconsin, has been elected president of the American Association for Cancer Research.

James H. Schulman has been named head of the Chemistry Branch of the Metallurgy Division of the Naval Research Laboratory. **Clifford C. Klick** is the new head of the Luminescent Section of the Chemistry Branch.

Romeo E. Short, director of the Foreign Agricultural Service, has been appointed chairman of the U. S.-UN Food and Agriculture Organization Inter-agency Committee. Mr. Short thus becomes the key official for relationships between the government and the United Nations FAO.

Kenneth W. Spence was awarded the Howard Crosby Warren Medal by the Society of Experimental Psychologists, for experimental work on fundamental problems of learning.

E. L. Tatum of Stanford University has been selected to present the 1953 Remsen Memorial Lecture of the American Chemical Society's Maryland Section.

Education

A plan to aid industry through fundamental research in engineering and science has been devised at **Carnegie Institute of Technology**. A regular and scheduled information service has been set up by the institution, making available to companies that give financial support for research to Carnegie reports on current research projects undertaken by individuals of the engineering and science departments. Also included are titles of scientific papers published by members of the departments in engineering and science journals during the past five years. Copies of doctoral theses which may be of interest to cooperating companies are also made available. A series of conferences, sponsored by the institution with representatives of business and industry in attendance, are presented by members of the engineering and science faculty. The representatives have the privilege of discussing projects with the scientists, and picking up ideas which might be applied in their own research and development projects.

The Amherst College herbarium of 85,000 specimens has been turned over to the Department of Botany of the **University of Massachusetts**. Under the new arrangement the director of the combined herbarium will be Theodore T. Kozlowski of the university. The basis of the present State Herbarium of the university was the W. H. Denslow collection of 12,000 sheets purchased in 1869. Recent accessions were derived from Williston Academy, Mass.; Auckland Museum, N. Z.; Duke University; Hawaii; Washington State College; Colombia; Argentina; Mexico; University of Texas; and the Philippines.

The **Mountain Lake Biological Station** of the University of Virginia in cooperation with the Biology Division, Oak Ridge National Laboratory, will offer a course in Radiation Biology at the station this summer. The course will run for five weeks (June 18-July 22) and will be divided into five sections: 1. Radiation Physics, by C. W. Sheppard; 2. Radiation Biochemistry, by J. R. Totter; 3. Radiation Genetics, by R. F. Kimball; 4. Radiation Cytology, by M. E. Gauden; 5. Radiation Biology of Microorganisms, by G. E. Stapleton. This is a new educational experiment, undertaken for the purpose of giving biology teachers and investigators a working acquaintance with radiation biology. A large cobalt source and other radiation equipment will be available for use. Costs for the course amount to \$116, including fees, room and board.

The **Social Research Foundation's Fund for Research in Psychiatry**, with headquarters at Yale University, has appointed Sibylle Escalona, assistant professor at the Yale Child Study Center, as Executive Officer. The Board of Directors, representing six leading medical schools throughout the country, is now working on the organization of the \$6,000,000 fund to support psychiatric research at universities and institutions

throughout the country. Frederick C. Redlich, professor of psychiatry at Yale and Chairman of the Board, explained that both income and principal of the fund will be expended over a period of about 20 years.

West Virginia University, with the cooperation of the Miles Laboratories Inc. of Elkhart, Ind., will undertake a three-year study of "The Relationship of Nutrient Intake to Nutritional Status in Human Subject Investigatorship."

Grants and Fellowships

The Department of the Air Force has awarded a contract to Case Institute of Technology for research over a two-year period on the heat treatment, properties, and theory of the behavior of high quality alloy steels at high-strength levels. The new contract calls for a doubling of the rate of effort and the technical scope of the investigation.

The following AAAS Research Grants have been awarded: South Carolina Academy of Science, to J. M. Rush, Clemson College, Clemson, S. C., for "A Study of the Morphological, Cultural, and Physiological Characteristics of the Bacteria of the Genus *Achromobacter*"; Kansas Academy of Science, to George E. Fay of Joplin, Mo., for "A Comprehensive Survey of Scientific Excavation of the Archaeological Culture of Sonora, Northwest Mexico," to L. J. Gier, William Jewell College, Liberty, Mo., for "A Study of Iowa and Missouri Mosses," and to Eugene Wittlake, University of Kansas, for "A Study of the Developmental Morphology and Phylogeny in the Marchantiales"; New Orleans Academy of Sciences, to Herman Sostrin, Department of Pharmacology, Tulane University, School of Medicine, for "The Isolation and Mode of Action of the Toxic Principle Responsible for Lathyrism, a Nutritional Paraplegic Syndrome"; St. Louis Academy of Science, to Hampton L. Carson and W. C. Blight of Washington University, for "Genetics and Ecology of Natural Populations of *Drosophila*."

Sharp & Dohme announces research grants to the Cardiorenal Fund of the Peter Bent Brigham Hospital, Boston: \$2500 for clinical investigation of the natriuretic compounds, Dirnate, and Dirnate analogs by John P. Merrill; to Northwestern University, a renewal grant of \$6000 for the work of Saul Malkiel on the mechanism involved in the production of hypersensitivity; to Tulane University, \$1200 for the study of Dirnate, Benemid, and pancreatic dornase by C. G. Collins; and to Cedars of Lebanon Hospital, Los Angeles, \$1800 for clinical research on Dirnate by Leonard M. Asher.

Recent research grants to Woman's Medical College, Philadelphia, include \$3500 from the Heart Association of Southeastern Pennsylvania to William G. Leaman, chairman of the Department of Medicine, for audiovisual aids in teaching in the Division of

Cardiovascular Diseases; \$2500 from Smith, Kline and French Laboratories to Jean Crump, professor of pediatrics, for basic research, and \$1500 from Smith, Kline and French to Nicholas Dreyer, professor of pharmacology, for equipment and research in the Department of Pharmacology.

In the Laboratories

The Los Alamos Scientific Laboratory of the University of California has made the following additions to its staff: W. W. Martin, of Sandia Corporation, Albuquerque, to the Chemistry and Metallurgy Division; L. K. Neher, from the University of California, to the Test Division; J. F. Torbert, of the Norfolk Naval Shipyard, Portsmouth, Va., to the GMX Division; E. M. Willbanks, of Arizona State College, to the Theoretical Physics Division; J. C. Anderson returns to the GMX Division; and B. D. McCarty, formerly of an oil-shale demonstration plant at Rifle, Colo., to the Metallurgy Division.

The U. S. Naval Ordnance Laboratory has made the following staff changes: A. E. Robertson, former deputy division chief of the Chemistry Division of the Engineering Department, has become chief of the Mechanisms and Services Division of the Fuze Department. Deputy chief of the Mechanisms and Services Division is now A. B. Dietemann, formerly of the Fuze Department Office. The Explosives Components group of the Chemistry Division has been transferred to Dr. Robertson's division in the Fuze Department as the Explosives Components Branch. The chief of this branch will be I. Kabik, who has been serving in a similar capacity in the Engineering Department. E. H. Beach has recently arrived at NOL to serve as branch chief of Branch No. 1 of the Electromagnetism Influence Division, Underwater Ordnance Department.

Recent staff changes at Oak Ridge National Laboratory include: C. E. Winters, acting director of the Reactor Experimental Engineering Division, has been appointed assistant research director; F. L. Culler has been named director of the Chemical Technology Division to succeed F. L. Steahly, who is leaving to become assistant manager of the Idaho Falls plant of the American Cyanamid Company.

A new experimental farm near Terre Haute, Ind., combining for the first time animal nutrition and veterinary medicine into a single research unit, was opened on April 14th by Chas. Pfizer & Co., Inc. Complete facilities including laboratories and offices are located in the center of a 700-acre tract formerly occupied by a Federal ordnance plant. Although the work of the two is closely coordinated, veterinary medicine and animal nutrition centers are separated to provide efficient disease control. The farm research team is headed by H. G. Luther, director of agricultural development for the Pfizer company, and James McGinnis, poultry nutritionist.

Meetings and Elections

The Golden Anniversary Meeting of the **American Leather Chemists Association** will be held in Cincinnati, Ohio, June 7-10. The program will feature both historical and technical sessions, highlighted by a symposium covering fifty years of leather-making.

The Eastern and New England Sections of the **Association of Geology Teachers** held a joint annual meeting at Rensselaer Polytechnic Institute, April 3-4. Officers of the Eastern Section for the coming year are: president, S. W. Lowman; vice president, G. F. Adams; secretary-treasurer, M. T. Heald; editor, Lawrence Whitcomb. Officers of the New England Section are: president, J. B. Lucke; vice president, C. G. Doll; secretary-treasurer, R. E. Stoiber.

The **Centre National de la Recherche Scientifique** in Paris announced two symposia organized with the help of the Rockefeller Foundation: "L'Etude des Molecules d'Eau Dans Les Solides au Moyen des Ondes Electromagnetiques," June 25-27, with W. Gordy, Duke University, and G. E. Pake, Washington University, St. Louis, as lecturers; and "Les Problemes Fondamentaux de la Classification Nucleaire," June 29-July 3, featuring A. J. Deutsch, Mount Wilson and Palomar Observatories, W. W. Morgan, Yerkes Observatory, and Bengt Stromgren, Yerkes Observatory.

At its fifteenth annual meeting, April 12-15 at White Sulphur Springs, W. Va., the **Industrial Research Institute** elected as its president Allen Abrams; H. G. Vesper was elected vice president and president-elect, and G. C. Worthington, secretary-treasurer.

A new society, the first of its kind in Venezuela, was founded in January. The **Sociedad Venezolana de Anatomia Patologica**, with headquarters in Caracas, aims to advance pathological research and to stimulate scientific relations among its members. Its first officers are: J. A. O'Daly, president; L. Potenza, vice president; and Alberto Rivero, secretary.

Miscellaneous

A tuberculosis "university" has been opened in Cairo to serve students from the Arab nations. It is being sponsored by the **Egyptian Ministry of Health** in association with the World Health Organization. The new center will train students in modern methods of tuberculosis control, both preventive and curative.

A systematic attempt to maintain the genetic corn stocks of the Corn Belt region will be inaugurated July 1 when a new corn genetics research center will be established at the **University of Illinois** under the supervision of M. M. Rhoades and J. R. Laughnan of the university's botany and agronomy departments. Earl Patterson of California Institute of Technology will serve as coordinator. Besides maintaining present genetic stocks, the new research center will develop new combinations of chromosomal testers,

determine linkage relations of unplaced genes, and search for new genes. States to be served under the new program will be Ohio, Indiana, Michigan, Wisconsin, Minnesota, North Dakota, South Dakota, Nebraska, Kansas, Missouri, Iowa, and Illinois. Co-operating with the university will be the U. S. Department of Agriculture Experiment Stations throughout the North Central region, and the Primary Introduction Station at Ames, Ia. The project will be supported by a grant from the U. S. Department of Agriculture.

The **Interamerican Foundation for Postgraduate Medical Education** has been organized for the purpose of encouraging exchanges of educators, postgraduate students, and research workers in the field of medicine and allied sciences in Latin and North American countries. Financial support has been obtained and more is now being solicited from firms in North America interested in the furtherance of friendly relations between the Americas. Committees of medical educators in each Latin American country will be asked to assume responsibility for nominating candidates for fellowships. The proposed program also provides for interchanges of a limited number of visiting lecturers, with expenses defrayed through the foundation. The Executive Director of the foundation is Alberto Chattas of Cordoba, Argentina. Present headquarters are at 112 E. Chestnut Street, Chicago 11, Ill.

The **Technical Information Division of the Library of Congress** (formerly the Navy Research Section) and the Document Service Center of the Armed Services Technical Information Agency (ASTIA), Dayton, Ohio, have been integrated to centralize report services for the Department of Defense and its contractors. ASTIA will perform indexing, abstracting, and comprehensive bibliographic services. The Document Service Center now receives technical documents and is the principal point of contact for technical reports services, including the publication of a high-speed *Title Announcement Bulletin*, which replaces the *Technical Information Pilot*, formerly published by the Technical Information Division. Also, the Center notifies users of the existence of new documents within a few days after their receipt by ASTIA. Library reference service is offered by ASTIA at the Document Service Center, the Technical Information Division, and the ASTIA regional offices in Los Angeles and New York.

Recent foreign visitors reported by the **National Bureau of Standards** include: Ole K. Anderson, Copenhagen; R. W. G. Hunt, Harrow, England; Stig Rahm, Grangersberg, Sweden; R. A. F. Hammond, Armament Research Establishment, Woolwich, England; H. A. B. Boulton, Cardiff, England; D. Nicholson, County Durham, England; H. Corsten-Moller, Copenhagen; Tore Siogvist, Boliden, Sweden; A. Perlat, Paris; Mario Dalmozzo, Corsica, Italy.

Technical Papers

Reversed Phase Paper Chromatography of Parathion and Related Phosphate Esters

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In our studies of the mode of action of parathion and related insecticides it became necessary to separate parathion, its S-phenyl and S-ethyl isomerides, and para-oxon (1, 2). After trials with paper chromatographic techniques using a variety of solvents and stationary phases, it was found that the separation could be accomplished by impregnating Whatman No. 1 filter paper with silicone 550¹ from a 5% hexane solution,² allowing it to dry, applying the phosphate esters in 5-10 μ l acetone solution, and employing ascending chromatography, using as solvent the upper phase from a mixture of 10 parts chloroform, 10 parts absolute ethyl alcohol, and 6 parts water. A similar technique was developed by Kritchevsky and Tiselius (3) for the separation of steroids. After chromatography, the position of the *p*-nitrophenyl esters was determined by converting them to the intensely yellow *p*-nitrophenate ion by spraying the paper with 5% alcoholic potassium hydroxide and heating in the oven for a few minutes at 105° C. By this means spots containing as little as 0.1 μ g of *p*-nitrophenol could be detected. The relative lengths of time required for the appearance of the yellow color are characteristic of the various phosphate esters and are determined by the alkaline hydrolysis constants as shown in Table 1. In general, the S-alkyl isomerides appear first, then the phosphates; heating is necessary to demonstrate the thionophosphates. Therefore, this characteristic can be used in determining the identity of unknown spots.

The described technique was applied to a variety of *p*-nitrophenyl dialkyl phosphate and thionophosphate esters and the R_F values shown in Table 1 were obtained. When the separations were carried out at a constant temperature with carefully impregnated paper, the R_F values were satisfactorily reproducible, as indicated by the standard errors for replicate determinations. In this connection it is important to impregnate the papers as evenly as possible, using a constant concentration of silicone.

When the chromatographic technique was applied to the separation of the constituents of technical parathion, three spots were consistently obtained with average R_F values of 0.04, 0.47, and 0.78. These appear to represent parathion, its S-ethyl isomeride, and *p*-nitrophenol, in ascending order. The percentages of the various constituents present can be estimated by cutting pieces of equal area around the spots, and then

¹ Dow Corning Corp.

² Skellysolve B.

TABLE 1
 R_F VALUES AND HYDROLYSIS CONSTANTS FOR PARATHION AND RELATED MATERIALS

Compound	R_F 26° C	First order K hyd min ⁻¹ 1M NaOH at 37° C
<i>p</i> -Nitrophenyl dimethyl phosphate	0.84 \pm 0.03*	7.7
<i>p</i> -Nitrophenyl diethyl phosphate	.74 \pm .06	1.7
<i>o</i> -Nitrophenyl diethyl phosphate	.74 \pm .09	1.7
<i>p</i> -Nitrophenyl di-2-chloroethyl phosphate	.68 \pm .03	
2,4-Dinitrophenyl diethyl phosphate	.64 \pm .03	
<i>p</i> -Nitrophenyl 0,8-dimethyl thiophosphate†	.58 \pm .03	
<i>p</i> -Nitrophenyl diisopropyl phosphate	.47 \pm .06	1.4
<i>p</i> -Nitrophenyl 0,8-diethyl thiophosphate†	.47 \pm .03	68
<i>p</i> -Nitrophenyl dimethyl thionophosphate	.14 \pm .01	0.69
<i>p</i> -Nitrophenyl diethyl thionophosphate	.04 \pm .01	0.23
<i>p</i> -Nitrophenyl ethyl thionobenzene phosphonate	.017 \pm 0.002	13.8
bis-(<i>p</i> -Nitrophenyl) ethyl thionophosphate	0.00	1.2

* Standard deviation.

† Determined from mixture.

dividing these into tiny pieces, which are eluted for 24 hr in 0.1 N NaOH in a 10-ml volumetric flask. After centrifugation, the *p*-nitrophenate ion in each sample is determined from the percentage transmission at 400 m μ as compared to a blank of filter paper and alkali. The method has also proved of value in demonstrating *p*-nitrophenol or other impurities in samples of the various aryl dialkyl phosphates or thionophosphates. The free *p*-nitrophenol can be distinguished by its yellow color, which is visible before treatment with alkali.

With this chromatographic method, it may be of value to determine the spots which contain phosphorus. The perchloric acid-ammonium molybdate technique of Hanes and Isherwood (4) is satisfactory for this purpose and is especially valuable with phosphate esters which contain no *p*-nitrophenyl group for identification. With this technique it is necessary to hydrolyze the esters on the paper for periods of 2-24 hr in a moist chamber at 85° C before developing the molybdenum blue color.

Two other techniques have been found useful to characterize the compounds resolved from complex mixtures. One consists in eluting the spots in acetone solution and determining the anticholinesterase activi-

ties of the compounds manometrically (5). In the other, the material is applied to the paper along 8 cm of the base line rather than as a spot and, after resolution, areas 8 x 5 cm containing the various compounds are cut from the paper and rolled in shell vials. Ten anesthetized houseflies are then introduced into each vial, and the toxicity of the compounds is characterized by rate of knockdown and 24-hr mortality.

The paper chromatographic method is useful in studying the metabolism of phosphorus insecticides in plants, mammals, and insects. With it, for example, we have been able to demonstrate the conversion of parathion and its methyl analog to the corresponding phosphates by an enzyme system found in *Periplaneta americana* (L.) (2). Further studies are in progress. The method has also been of value in studying the action of heat on purified parathion and methyl parathion and in isolating the compounds formed and in studying their biological properties (1).

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A Production of Amino Acids Under Possible Primitive Earth Conditions

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The idea that the organic compounds that serve as the basis of life were formed when the earth had an atmosphere of methane, ammonia, water, and hydrogen instead of carbon dioxide, nitrogen, oxygen, and water was suggested by Oparin (1) and has been given emphasis recently by Urey (2) and Bernal (3).

In order to test this hypothesis, an apparatus was built to circulate CH_4 , NH_3 , H_2O , and H_2 past an electric discharge. The resulting mixture has been tested for amino acids by paper chromatography. Electrical discharge was used to form free radicals instead of ultraviolet light, because quartz absorbs wavelengths short enough to cause photo-dissociation of the gases. Electrical discharge may have played a significant role in the formation of compounds in the primitive atmosphere.

The apparatus used is shown in Fig. 1. Water is boiled in the flask, mixes with the gases in the 5-l flask, circulates past the electrodes, condenses and empties back into the boiling flask. The U-tube prevents circulation in the opposite direction. The acids

¹ National Science Foundation Fellow, 1952-53.

² Thanks are due Harold C. Urey for many helpful suggestions and guidance in the course of this investigation.

and amino acids formed in the discharge, not being volatile, accumulate in the water phase. The circulation of the gases is quite slow, but this seems to be an asset, because production was less in a different apparatus with an aspirator arrangement to promote circulation. The discharge, a small corona, was provided by an induction coil designed for detection of leaks in vacuum apparatus.

The experimental procedure was to seal off the opening in the boiling flask after adding 200 ml of water, evacuate the air, add 10 cm pressure of H_2 , 20 cm of CH_4 , and 20 cm of NH_3 . The water in the flask was boiled, and the discharge was run continuously for a week.

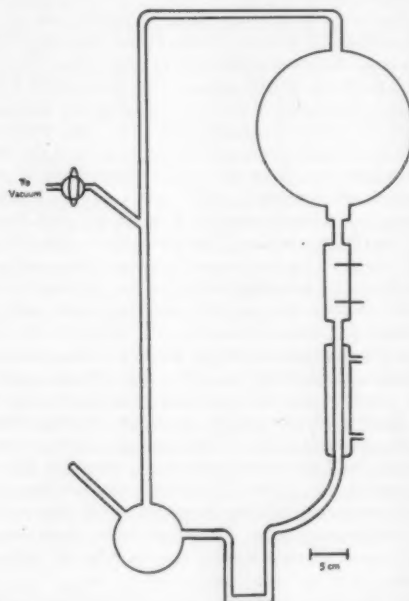


FIG. 1.

During the run the water in the flask became noticeably pink after the first day, and by the end of the week the solution was deep red and turbid. Most of the turbidity was due to colloidal silica from the glass. The red color is due to organic compounds adsorbed on the silica. Also present are yellow organic compounds, of which only a small fraction can be extracted with ether, and which form a continuous streak tapering off at the bottom on a one-dimensional chromatogram run in butanol-acetic acid. These substances are being investigated further.

At the end of the run the solution in the boiling flask was removed and 1 ml of saturated HgCl_2 was added to prevent the growth of living organisms. The ampholytes were separated from the rest of the constituents by adding $\text{Ba}(\text{OH})_2$ and evaporating *in vacuo* to remove amines, adding H_2SO_4 and evaporat-

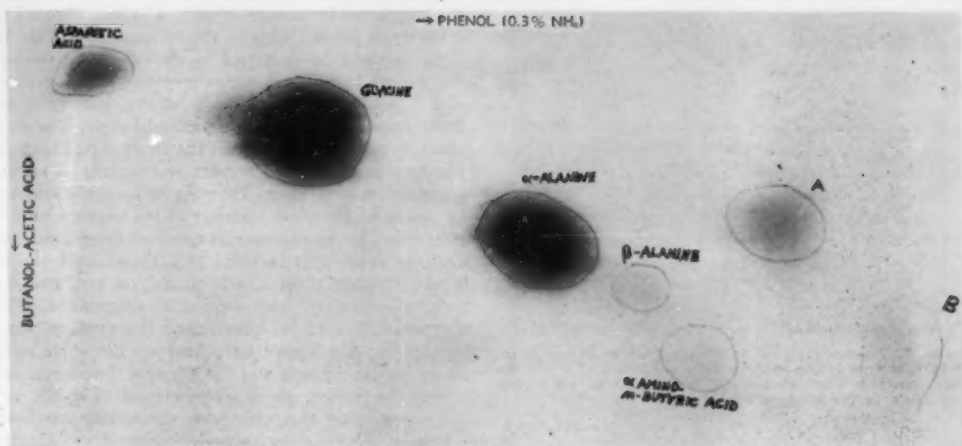


FIG. 2.

ing to remove the acids, neutralizing with $\text{Ba}(\text{OH})_2$, filtering and concentrating *in vacuo*.

The amino acids are not due to living organisms because their growth would be prevented by the boiling water during the run, and by the HgCl_2 , $\text{Ba}(\text{OH})_2$, H_2SO_4 during the analysis.

In Fig. 2 is shown a paper chromatogram run in *n*-butanol-acetic acid-water mixture followed by water-saturated phenol, and spraying with ninhydrin. Identification of an amino acid was made when the R_f value (the ratio of the distance traveled by the amino acid to the distance traveled by the solvent front), the shape, and the color of the spot were the same on a known, unknown, and mixture of the known and unknown; and when consistent results were obtained with chromatograms using phenol and 77% ethanol.

On this basis glycine, α -alanine and β -alanine are identified. The identification of the aspartic acid and α -amino-*n*-butyric acid is less certain because the spots are quite weak. The spots marked A and B are unidentified as yet, but may be beta and gamma amino acids. These are the main amino acids present, and others are undoubtedly present but in smaller amounts. It is estimated that the total yield of amino acids was in the milligram range.

In this apparatus an attempt was made to duplicate a primitive atmosphere of the earth, and not to obtain the optimum conditions for the formation of amino acids. Although in this case the total yield was small for the energy expended, it is possible that, with more efficient apparatus (such as mixing of the free radicals in a flow system, use of higher hydrocarbons from natural gas or petroleum, carbon dioxide, etc., and optimum ratios of gases), this type of process would be a way of commercially producing amino acids.

A more complete analysis of the amino acids and other products of the discharge is now being performed and will be reported in detail shortly.

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A Vacuum Microsublimation Apparatus

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The analytical biochemist is frequently confronted with the task of isolating microquantities of substances in a chemically pure state from small quantities of tissues or biological fluids. Kofler (1) edited a book covering the use of microsublimation, melting point, eutectics, etc., in identifying microquantities of organic material. The advantages of sublimation over other methods of purification have been discussed by Hubacher (2). Many types of vacuum sublimation apparatus have been described (1-3). The equipment described here is inexpensive and can be assembled readily by any laboratory worker with a modicum of glassblowing skill.

To a thick-walled, round-bottom, Pyrex test tube, 30 x 200 mm, is attached a glass side arm about one in. from the bottom. Using a suspension of very fine emery in glycerin or fine valve-grinding compound, the open end of the test tube is ground against the aluminum block of a Fisher-Johns melting point apparatus (Fisher Scientific Co., St. Louis, Mo.) until it makes a vacuum-tight seal when dry. This is the vacuum hood. Microbeakers are prepared from flat-

¹ The author is indebted to Robert Puckett, of this laboratory, for technical assistance in preparing this apparatus.

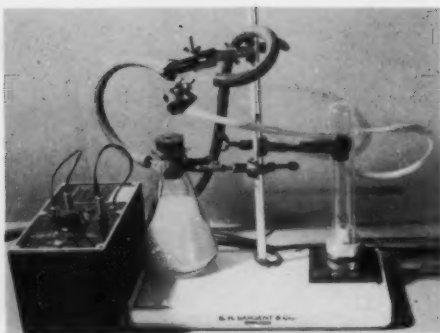


FIG. 1. Vacuum microsublimation apparatus in operation. The vapor trap in the vacuum line, consisting of a vacuum flask partially filled with glass beads, aids in preventing turbulence in the vacuum hood.

bottom test tubes, 15 mm in diameter. These may be cut down to any height, depending on the capacity desired (10 mm in height is equivalent to about 1 ml in capacity). Commercially available 5-ml beakers also may be used.

The biological fluid, tissue extract, or solution is concentrated by evaporation to 1 or 2 ml and then transferred to a microbeaker. The beaker is placed on the heating block of the melting point apparatus and heat applied. The ground-glass lip of the vacuum hood is then placed over the beaker on the block. Vacuum is applied to assist in drying the residue.

When the residue is completely dry the vacuum hood is removed, and the microbeaker is encased in aluminum foil. This prevents sublimation from occurring on the walls by keeping the wall temperature the same as that on the bottom. A microscope cover slip is now placed over the mouth of the microbeaker, and the vacuum hood is placed over this assembly on the heating block. The hood is pressed down on



FIG. 2. Micromelting point apparatus with heating block mounted on stage of dissecting microscope. Contrast between crystals and background is enhanced by coating the heating block surface with a black, heat-stable substance such as ceramic ink.

the block and vacuum slowly applied through a screw-type pinch clamp in the vacuum line (Fig. 1). Caution must be exercised in applying the vacuum to prevent turbulence, which may displace the cover slip. The temperature of the block is gradually raised by means of a voltage regulator until crystals of sublimation are seen forming on the cover slip. This temperature is maintained for a few moments to ensure quantitative separation. The vacuum is slowly released by means of the pinch clamp, and the vacuum hood is removed. The microbeaker is removed from the block and the cover slip inverted and transferred to the block. Another cover slip is placed on top, and the melting point of the sublimate determined. For sharper definition in determining the melting point the heating block may be placed on the stage of a dissecting microscope and the crystals thus magnified during the melting point determination (Fig. 2).

By employing this apparatus the author has been successful in quantitatively recovering 4 μ g Amytal from 2.5 ml of whole blood.

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The Effect of Carbon Monoxide on Respiration in the Sweet Potato, *Ipomoea batatas* Lan.¹

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Since the addition of certain *ortho*-diphenolic substances (catechol, chlorogenic acid, etc.) to thin slices of roots of sweet potatoes increases the rate of oxygen consumption and carbon dioxide evolution, it has been suggested that a polyphenol oxidase is the terminal oxidase in sweet potato respiration (1, 2). In line with this, Walter (3) reported that sweet potato homogenates showed considerable phenol oxidase activity, but little or no cytochrome oxidase activity. This has been interpreted as further evidence for the possible operation of the phenol oxidase as a terminal oxidase, since the apparently insignificant amounts of cytochrome oxidase that were found could hardly play a major respiratory role in this tissue (4).

Recently, however, the author was able to demonstrate a very active cytochrome oxidase in sweet potatoes by a spectrophotometric method (5). It was also shown that crude sweet potato homogenates are not

¹ The author wishes to thank Albert W. Frenkel, of the University of Minnesota, for his helpful comments.

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suitable for the manometric estimation of cytochrome oxidase by the technique used by Walter (3), because the high phenol oxidase activity of these preparations masks other oxidative activity in the same system. Distinct cytochrome oxidase activity could be demonstrated manometrically if the homogenates were dialyzed thoroughly, or if the particulate cellular fraction (containing the cytochrome oxidase activity) was separated from the phenol oxidase by high-speed centrifugation.

Therefore, since a cytochrome oxidase is also present in sweet potatoes, and since the elicitation of increased gas exchange on the addition of phenolic substances does not seem to be a very critical basis for determining the nature of a terminal oxidase in living cells, it was of interest to re-examine the role played by phenol oxidase in sweet potato respiration. For this purpose, the effect of carbon monoxide on respiration was studied, as it had been shown earlier (6) that the carbon monoxide inhibition of sweet potato phenol oxidase activity was insensitive to light, whereas the carbon monoxide inhibition of sweet potato cytochrome oxidase was easily eliminated by light. The respiration of thin disks (7 mm diam, 0.5 mm thick) of root tissue in 0.05 M potassium phosphate buffer (pH 7.1) was measured at 25° C by standard manometric techniques (7) in various ratios of carbon monoxide or nitrogen to oxygen. The system was irradiated with light of about 300 ft-c incident on the manometer vessels.

In Table 1 are given values for oxygen consumption by sweet potato disks in several gas mixtures and in darkness and light. Respiration was strongly inhibited at the higher ratios of carbon monoxide to oxygen. It is evident that the inhibitions were completely eliminated by illumination except for a small amount at the highest CO/O₂ ratio. For comparison, the results with purified preparations of both sweet potato cytochrome oxidase and phenol oxidase under the same conditions are also presented.

Several varieties of sweet potatoes were examined, but no essential differences in results were noted that were due to difference in variety. Inasmuch as the

carbon monoxide inhibition of respiration was so sensitive to light, and the carbon monoxide inhibition of phenol oxidase activity was completely insensitive, it would seem that sweet potato phenol oxidase is precluded from consideration as a terminal oxidase of respiration under the conditions used here. Conversely, the participation of the cytochrome oxidase in respiration, although not entirely proved, is rendered likely by the similar reaction of respiration and of cytochrome oxidase activity to carbon monoxide inhibition and to illumination.

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The Dependence of the Secondary Sex Ratio in Humans on the Age of the Father¹

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That older mothers produce fewer male offspring than younger mothers is well known. This decrease in sex ratio with increasing age has given rise to a number of conjectures, some of which are based on embryological or gynecological considerations (1-3). From the genetic point of view, one might be inclined to wonder if possibly this decrease in sex ratio is, in fact, a function of the age of the father, with the above relationship being a simple consequence of the correlation between ages of spouses.

A simple statistical test has been made of data bearing on this point. From the analysis described below, it appears that this decrease in sex ratio is actually linearly related to the age of the father and is independent of the age of the mother.

The appropriate statistics giving the sex of each child at birth, with the ages of the mother and of the father, are given in the yearbooks of the U. S. Bureau of Vital Statistics for the years 1947, 1948, and 1949. The figures for the three years for the whites have been combined, including only those births for which the ages of both parents were known.

A multiple linear regression has been calculated for these data. Only those aspects of the calculations bearing on the general argument and the tests of significance will be presented here.

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TABLE 1
EFFECT OF CARBON MONOXIDE ON OXYGEN CONSUMPTION BY SWEET POTATO SLICES, BY PURIFIED PHENOL OXIDASE, AND PURIFIED CYTOCHROME OXIDASE FROM SWEET POTATO, AND THE EFFECT OF LIGHT ON THE OBSERVED INHIBITION

Experimental material	Gas ratio (in the liquid phase)	Percentage inhibition of oxygen consumption in a given ratio of CO/O ₂ as compared with a control in N ₂ /O ₂ of the same ratio	
		In the dark	In the light
Slices	3.1	25	0
"	6.8	46	0
"	14.4	67	12
Phenol oxidase	6.8	80	81
Cytochrome oxidase	6.8	68	0

Following the usual convention in approaching problems of multiple linear regression (4), the best fitting plane is defined as

$$Y = a + b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2),$$

where Y = calculated sex ratio for a given set of ages of parents, a = average sex ratio, b_1 = regression coefficient on the age of the father, b_2 = regression coefficient on the age of the mother, x_1 = age of the father, in units of five-year intervals, with the 25-29 age group defined as the 0 class, x_2 = age of the mother, defined similarly to x_1 , and \bar{x}_1 , \bar{x}_2 = average ages of fathers and mothers, respectively, at the time of birth of children.

The calculation of a , b_1 , and b_2 gives the equation

$$Y = 0.5142367 - .000629 (x_1 - \bar{x}_1) - .000196 (x_2 - \bar{x}_2).$$

The standard errors of the constants are:

$$\begin{aligned} s_a &= .000164 \\ s_{b_1} &= .000173 \\ s_{b_2} &= .000202 \end{aligned}$$

The analysis of the sums of squares gives:

Item	χ^2	N
Regression	42.5	2
Remainder	84.8	87
Total	127.3	89

The ratio b_1/s_{b_1} equals 3.6, which corresponds to a P -value of less than .001. However, the ratio $b_2/s_{b_2} = 0.97$, with P -value of 0.3. Thus it appears that there is highly significant effect of the age of the male, but not of the age of the female.

Assuming that b_2 is in fact equal to zero, a simple linear regression may be calculated, which gives the following equation:

$$Y = 0.5142367 - .0007535 (x - \bar{x}_1).$$

The analysis of the sums of squares gives:

Item	χ^2	N
Regression	41.6	1
Remainder	5.8	8
Total	47.2	9

The low value of the remainder χ^2 , although adding very little to the evidence provided by the partial regression coefficients, shows excellent agreement of the data with the hypothesis that the sex ratio is adequately accounted for by simple linear regression on the age of the father.

E. R. Dempster, of the University of California, has analyzed the above data using the path coefficient method of Wright (5), from which it can be estimated that about 23% of the variance in sex ratio is due to a direct effect of the age of the father, 1.6% to a direct effect of the age of the mother, and 9% to a joint effect, the latter two, however, not being statistically significant.

A multiple regression has also been calculated for comparable data for the nonwhite population. The values of the constants are as follows:

$$\begin{aligned} a &= .50630 & s_a &= .00046 \\ b_1 &= -.00074 & s_{b_1} &= .00040 \\ b_2 &= +.00033 & s_{b_2} &= .00161 \end{aligned}$$

Neither of the coefficients differs significantly from zero. It should be pointed out, however, that the total number of nonwhite births for which information on parental ages was available for the three-year period was 1,157,994, whereas the white births amounted to 9,279,697. It does not seem unlikely that additional data will lead to the same conclusion for the nonwhite births as for the white births; i.e., that there is a decrease in sex ratio independent of the mothers' age but dependent on the fathers' age.

Finally, it must be noted that the over-all sex ratio, a , is significantly lower in the nonwhites than in the whites, in agreement with previous comparisons of this kind. This cannot be accounted for by a lower average age of nonwhites at the time of birth of offspring. The average age of white fathers is 0.588 (or slightly more than half of a 5-year unit above the midpoint of the 25-29 year age group) and of the nonwhite fathers, 0.564. This lower age could account for a smaller percentage, at most, of the observed difference.

The conclusion that the changing age of the male, rather than of the female, parent influences the sex ratio does not necessarily mean that a genetic mechanism must be involved. There might be conditions of, for instance, a physiological nature which, changing with the age of the male, would tend to shift the sex ratio. This analysis does indicate, however, that such forces would have to operate on the male sex predominantly, if not exclusively, and, contrarily, those explanations of the shift based on factors affecting the female sex would seem to be excluded. It might be pointed out, also, that this age effect could very well be responsible for other correlations that have been previously established. One such is the decreasing sex ratio with increasing birth order, studied by Cioeco (6). His data give the percentage of males among first-born as 0.5153, and among the fifth or higher order as 0.5124. An average age difference of fathers amounting to only about twenty years would be required to account for this difference. It would be instructive to analyze data giving simultaneously the sex of the child, birth order, and age of the father, if such data were available.

This shift in the sex ratio is superimposed on an average sex ratio which, as is well known, deviates significantly from the simple 1:1. Whether this average deviation is likewise primarily a function of the male sex is a highly controversial question. The simplest genetic considerations suggest that X- and Y-bearing spermatozoa should be produced in exactly equal numbers. It is conceivable, however, that this is not so; one hypothetical system which would allow for a certain amount of deviation has been described by the author (7). With the limited information available at present, this system is purely conjectural, and

consequently no detailed consideration will be given it here. It might be pointed out, however, that this mechanism, based primarily on the nonrandom disjunction of heteromorphic homologues in *Drosophila*, would adequately account for the age effect described here by attributing it to a decreasing level of crossing over with increasing age and would also account for interracial differences as a reflection of the degree of structural heterozygosity in those races.

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Hibernation and Cortical Electrical Activity in the Woodchuck (*Marmota monax*)¹

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Previous work from these laboratories showed that spontaneous cortical electrical activity could not be recorded in the golden hamster arousing from hibernation until the cortical temperature had reached 19°–21° C (1). These studies, extended to the woodchuck (*Marmota monax*), reveal profound differences between this hibernator and the hamster in regard to the electrocorticogram and in the behavior of the animal during hibernation.

In the present experiment the electrocorticogram and the cortical temperature were recorded by means of a device that had been previously attached to the skull. This device was made throughout of stainless steel and consisted essentially of a plate 1×1×0.1 cm, which was drilled at each corner to receive screws and drilled and threaded in the center to receive the electrode and thermocouple carrier. The carrier was a short, L-shaped tube of 0.15 cm outside diameter, threaded on one end to screw into the central hole of the plate, and containing 2 silver electrodes and an iron-constantan thermocouple. The electrodes protruded slightly from the end of the threaded tube, and the inside of the tube was filled with polyethylene.³ All wires were coated with the same substance.

To attach the device, the skull of a woodchuck, which was hibernating in a cold room kept at 3°–7° C, was exposed under sterile conditions, and the plate fastened

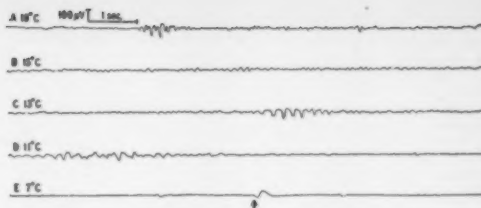


FIG. 1. Electrocorticograms of a hibernating woodchuck at various cortical temperatures as indicated. The arrow in record E indicates a cortical response evoked by noise. Other deflections in record E are artifacts from EKG. Calibration in record A applies to all.

to the skull by the screws. A hole was then drilled in the skull through the central opening of the plate and the carrier screwed in place so that the electrodes rested on the dura. The wires from the thermocouple and electrodes were forced through the subcutaneous tissue and brought out through the skin of the interscapular region. The incision in the skin of the head was then closed over the device. Cortical temperature was measured by a Micromax thermoelectric recorder. The operative procedure, which did not require anesthesia because the animal was hibernating, caused the animal to awaken gradually with a concurrent increase in body temperature. Five days later, when it was observed that the animal was re-entering hibernation, recording of cortical activity (on a Grass ink-writer) was started and continued intermittently for 18 days. During this period the animal's cortical temperature fluctuated between 6° and 35° C.

Since a cortical response could easily be evoked by auditory stimulation, even at cortical temperatures as low as 7° C, it was concluded that the electrodes had been placed on an auditory receiving area (Fig. 1 E). Slow, nondescript, spontaneous cortical activity could also be recorded at this temperature, although it was sporadic. At 11° C and above, spontaneous burst activity was apparent, and the components of the bursts increased in frequency as the temperature rose (Fig. 1 A–D). Adequate recordings at temperatures higher than 18° C were precluded by the large number of muscle artifacts caused by the tensing and movement of the animal.

These observations are reported at this time because Kayser and his co-workers (2, 3) have recently recorded spontaneous cortical activity in the hibernating and artificially cooled ground squirrel at deep body temperatures of 5°–6° C and have commented upon how different their results are from those described by us in the hamster, without suggesting that species differences might explain the discrepancy. Like the ground squirrel, the woodchuck shows spontaneous cortical electrical activity at body temperatures at which it is completely absent in the hamster. Furthermore, the woodchuck shows an evoked auditory cortical potential at temperatures at which the auditory nerve of the hamster does not conduct (4). The general pattern of hibernation in the woodchuck also differs

¹ This research was supported by a grant under Air Force Contract AF 33(038)-18133.

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³ Clay-Adams Co., Inc., New York.

profoundly from that of the hamster, as the woodchuck is capable of responding to auditory and mechanical stimulation by moving about at body temperatures at which the hamster is completely immobile.

The recorded differences in the electrocorticogram of the hibernating ground squirrel and woodchuck as contrasted with the hamster emphasize that generalizations about physiological processes which occur in hibernating mammals should be guarded and that the species should always be indicated.

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Observations on a Class of Free Radicals Derived from Aromatic Compounds¹

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The reaction of alkali metals with aromatic hydrocarbons has been under investigation for many years. It was not until 1935, however, when it was found that ethers such as methyl ether or 1,2-dimethoxyethane promote the reaction (1, 2), that significant progress was made toward an understanding and utilization of the reaction.

It has been suggested (2-5) that the reaction of an alkali metal with an aromatic hydrocarbon in the presence of one of the above ethers involves the transfer of either one or two electrons from alkali metal atoms to one molecule of hydrocarbon. We have found that the intensely colored substances formed upon reaction of sodium with naphthalene, anthracene, naphthacene, 1,2-benzanthracene, 20-methylcholanthrene, nitrobenzene, *m*-dinitrobenzene, 1,3,5-trinitrobenzene, or 2,3,7-trinitrofluorenone, in 1,2-dimethoxyethane or tetrahydrofuran as solvents, exhibit intense paramagnetic resonance absorptions.^{2, 3} In none of these paramagnetic products does the gyromagnetic ratio deviate from the free electron value by more than a few tenths of 1%. It is difficult to reconcile these observations with the suggestion that the reaction involves the transfer of two electrons from two atoms of sodium to one molecule of aromatic compound. This hypothesis would require that in each case the normal state of these

paramagnetic molecules is a triplet (biradical) electronic configuration. Such a situation is highly unlikely in molecules in which orbital degeneracy does not exist. All the molecules under discussion here belong to the class in which no orbital degeneracy is permitted.

These considerations, in addition to the fact that the over-all reaction involves one aromatic molecule per atom of sodium (1, 2), indicate that we are dealing with free radical ions formed by the transfer of one electron to the aromatic compound. The reaction, using naphthalene as an example, may be represented by the equation:



The analogy between this equation and the equations for the solution of sodium in liquid ammonia and triphenylmethyl in liquid sulfur dioxide is worth noting.

Scott, Walker, and Hansley (2) demonstrated that the reaction is an equilibrium. They also pointed out that methyl ethers, such as dimethyl ether or 1,2-dimethoxyethane, are very effective in shifting the above equilibrium to the right, whereas ethyl ether, for example, is relatively ineffective. It is believed that, in general, it is the magnitude of the energy of solvation of the metal ion (and perhaps also the negative hydrocarbon ion) by the ether which is the principal factor in determining the value of the equilibrium constant for the above reaction. It then follows that ethers which are relatively unhindered sterically (6), or polyethers which can form chelate structures with the metal ion (4), will be most effective in shifting the above equilibrium to the right. This conclusion is in agreement with the qualitative experimental data that are available.

The shape of the paramagnetic resonance absorption curve is dependent on the particular free radical and on its concentration. The hydrocarbon free radicals at concentrations in the neighborhood of 10^{-4} *M* yield a single absorption band about 5 oersteds wide. As the concentration is increased from this value, the band width decreases. Absorption bands narrower than 1 oersted are observed at high concentrations (ca. 0.1 *M*). The only mechanism thus far suggested for such a narrowing involves an exchange of spins between free radical molecules, the exchange proceeding because of orbital interaction (7). The fact that this narrowing is observable at concentrations of the order of 10^{-3} *M* indicates that the orbital interaction in these molecules may be exercised at large distances.⁴

The paramagnetic absorption spectra of the nitro-free radicals, with the exception of the fluorenone derivative, exhibit remarkable structures in dilute solutions. From 1,3,5-trinitrobenzene a free radical is obtained whose absorption shows 8 symmetrically distributed, evenly spaced peaks. Nitrobenzene and *m*-dinitrobenzene each yields a free radical whose absorption has 10 peaks; the relative intensities of the peaks in each pattern are different for the two compounds. The absorption in each case covers about 25 oersteds.

⁴The average separation between molecules at 10^{-3} *M* is about 100 Å.

¹ Assisted by the joint program of the Office of Naval Research and the Atomic Energy Commission.

² Our observations were made at 9000 megacycles/sec in fields in the neighborhood of 3200 oersteds.

³ We are indebted to the U. S. Industrial Chemicals Co., Division of National Distillers Products Corporation, for the sodium dispersion used in many of our experiments.

It is probable that the observed spectral structures are to be classed as hyperfine—they arise from interactions between the magnetic moments of the nitrogen and hydrogen nuclei in each molecule with the magnetic moment of the odd electron. Despite the complexity and breadth of the spectra of their dilute solutions, the crystals of the nitro-free radicals exhibit a single sharp line.⁵ The trinitrofluorenone derivative yields a rather broad, unresolved resonance peak. It is possible that the fine structure in this case, as in the case of many other free radicals, is unresolved because of its complexity. This complexity arises from the large number of nuclear moments with which the electronic moment interacts.

Although the fact that sodium metal catalyzes the polymerization of unsaturated compounds has been known for a long time, there is no general agreement concerning the mechanism of this catalysis (8). Two sets of experiments have been performed with styrene which indicate that the function of the sodium metal is to bring about the formation of a negative hydrocarbon free radical. This free radical ion then initiates the chain reaction involved in the polymerization, as suggested by Bolland (9) for the sodium-catalyzed polymerization of isoprene. In one experiment a small amount of sodium dispersion was added to a sample of styrene. After some hours the styrene had been converted to a deep-red, rubbery solid which showed a strong paramagnetic resonance absorption. It is believed that the red color and the paramagnetic absorption are due to species such as $(C_6H_5CH=CH_2)^-$ which were trapped in the polymer. The material retained its paramagnetic resonance absorption and its color over a period of several months.⁶ In other experiments, styrene was dissolved in 1,2-dimethoxyethane and a small amount of sodium dispersion was added. A very vigorous reaction set in immediately, accompanied by the formation of a deep-orange color. As the reaction subsided, the reaction mixture became very viscous and the orange color gradually faded away. Since 1,2-dimethoxyethane, as mentioned above, favors the formation of negative hydrocarbon free radicals, it is believed that these observations are further evidence that the actual polymerization catalyst is a species such as $(C_6H_5CH=CH_2)^-$.

Various investigators have suggested (10) that abnormal growth may be explained by a free radical mechanism. Perhaps the carcinogenic activity of 20-methylcholanthrene and 1,2-benzanthracene is due to their ability to form negative hydrocarbon free radicals with mild reducing agents, whereas noncarcinogenic hydrocarbons such as naphthalene and anthra-

cene are able to form such free radicals only with very strong reducing agents.⁷

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⁷ A study of the relative electron affinity of various hydrocarbons is in progress in this laboratory.

Excessive Intake of Vitamin A as a Cause of Congenital Anomalies in the Rat¹

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Congenital anomalies have been produced in animal young when the fetal environment has been influenced by chemical, endocrine, mechanical, and actinic factors (1). Changes in atmospheric pressure (2) (hypoxia) and administration of cortisone (3) have recently been shown to exert teratogenic effects. Similarly, deficiencies in the maternal diet of single nutritional elements such as minerals (copper [4] and iodine [5]) and vitamins (riboflavin [6], pantothenic acid [7], folic acid [8], and vitamin A [9, 10]) have induced defective offspring. Reports (11, 12) of vitamin A excess in the maternal diet have shown a diminished litter rate and a high incidence of fetal resorption *in utero*. In the course of our investigation of the skeletal changes of hypervitaminosis A in mature rats, it was noted that several pregnant animals produced offspring with congenital malformations. An investigation was undertaken to study this phenomenon.

One hundred and fifty female rats of the CF Wistar strain (175-200 g) were mated by exposure for 24 hr, during the pre-ovulatory stage, to males of the same strain. Pregnant females were fed the standard Rockland pellet diet and water *ad lib*. From the 2nd, 3rd, or 4th to the 16th day post coitus, 35,000 IU vitamin A in 0.7 ml diluent² were administered daily, via stomach tube, to 100 animals in the experimental group.

¹ Aided by grants from the Loyal League for Philanthropies, Inc., New York City, and Mead Johnson Co., Evansville, Ind.

² The vitamin product used was an aqueous preparation containing 50,000 USP u/cc natural vitamin A dispersed in sorbitan monolaurate and water.

TABLE 1
INCIDENCE OF CONGENITAL CRANIAL ANOMALY

Animal no.	No. of offspring in litter	No. with congenital cranial anomaly	Gestation days fed 35,000 IU vitamin A
14	10	4	2-16
30	6	4	4-16
35	6	4	4-16
39	6	1	4-16
59	9	5	3-18
62	11	3	3-16
79	9	9	2-16
90	2	2	2-16
93	5	5	2-16
100	8	2	2-16
Total	72	39 (54%)	

Fifty control animals were fed 0.7 ml of the diluent. The animals were sacrificed on the 20-21st day post coitus (at or near term), and each litter was ex-

skull and brain, a deformity rate of 54%. In the control group no anomalies were noted in 410 offspring.

The gross developmental defect apparent in each of the abnormal offspring rats is an extrusion of the brain (Fig. 1) to the external surface of the head. There is a thin membranous covering over the exposed brain tissue. Sporadic anomalies noted were macroglossia, harelip, cleft palate, and gross defects in eye development. The cranial deformity, however, was a consistent finding. It is of interest that comparable cranial anomalies (as determined by photographic similarity) have been produced in mice by other teratogenic methods such as hypoxia (2) induced by exposure of the pregnant animal to atmospheric pressures of high altitudes, or by the translocation effect (13) in the second-generation offspring following irradiation of the initial sire male.

Results of this preliminary experiment indicate that the administration of excessive amounts of vitamin A to pregnant rats produces a diminished litter rate and

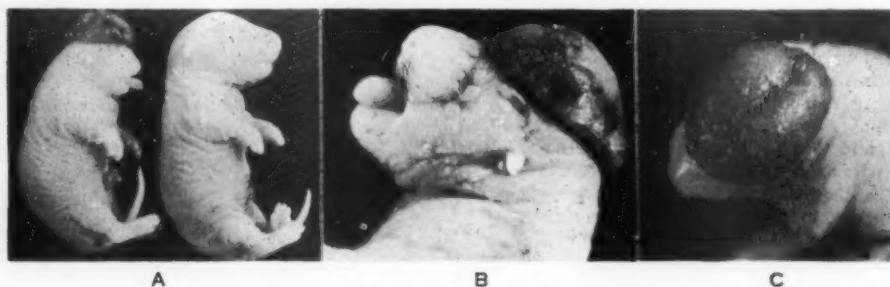


FIG. 1. A, lateral view of experimental and control rat at term. B, lateral close-up of experimental animal showing brain extruded to surface of head. Protruding tongue is abnormal. C, close-up view of extruded brain from above. Note longitudinal fissure of the cerebrum.

amined for gross congenital defects. In both groups most of the young were alive when removed from the amniotic sac. All were placed in fixative solution for histologic study.

Excessive intake of vitamin A from the 2nd, 3rd, or 4th to the 16th day of gestation resulted in a marked reduction in the number of litters carried to term. Of 50 control mated females, 44 produced litters, with a total of 410 apparently normal newborn, a successful pregnancy rate of 88%; whereas of 100 mated females in the experimental group, only 10 carried young to term, with a total of 74 offspring, a successful pregnancy rate of 10%. This marked pregnancy failure rate is in accord with previous reports (11, 12).

The incidence of gross congenital defects in the litters carried to term in the experimental group is seen in Table 1. Of 74 offspring produced in 10 litters, 34 exhibited a gross anomaly in the development of the

characteristic malformations among the surviving young.

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Manuscript received September 24, 1952.

Comments and Communications

Addenda to Disa and Data

THERE is another solecism that needs dissection at the same time that "data" is being given its proper treatment, and that is the wrong use of Latin plurals in hyphenated compounds. The type of this species of error may be taken to be "bacteria-like colonies." No one would write "trees-like plants" or "oysters-like mushrooms"; but when the name of the object to which another is likened happens to be a Latin one, the incongruity of a plural escapes the author and, too often, the editor. It seems clear that in the specimen under consideration "bacteria" is considered a singular noun, as was "curricula" by a writer in one of our literary journals (1) who deplored "curriculae" loaded with science courses as productive of graduates unable to use good English.

"Ik," incorrectly used as though it meant clan or kind, and "internecine," falsely interpreted as though analogous to "intramural" and similar in sense to "fratricidal," are literary vogue-words as impossible for scientists and Fowler's *Modern English Usage* to suppress as is "intrigue," used to mean fascinate. Scientists do, however, work with hypotheses; they may assume a condition or, as a poor second choice, may hypothesize. But the occasional theorist who tries to hypotheate a hypothesis should be put on warning that he is attempting to pledge it as security, and that no pawnbroker is likely to attach much value to it.

DONALD P. ROGERS

The New York Botanical Garden

Reference

1. *Sat. Rev. Lit.*, 32, (49), 20 (1949).

AFTER (not "following") the remarks of Frank C. Calkins (*SCIENCE*, 115, 486 [1952]) and the letter from S. Reid Warren, Jr., that (not "which") you published before (not "prior to") it (*SCIENCE*, 115, 633 [1952]), as well as your welcome editorial comments about (not "regarding") the word "data," you may perhaps allow me to add a word on the dilemma of deciding between common usage and etymological probity. For this I take to be the guts of the dispute, if there be one any longer. The dilemma can often be avoided (incidentally, the proper treatment of dilemmas) by refraining from using the word "data" in the wrong sense, as it generally is used.

I think your readers, like many others, may be ignoring a communication by A. V. Hill (*Nature*, 164, 410 [1949]) in which he pointed out that "data" and "results" are not synonymous. Although, as I wrote after Professor Hill's remark, I am prepared to give a little more scope to the word than he is; I am entirely with him in rejecting the use of the term when "results," "figures," or some other word would indicate, as the word "data" does not, that the things in question have been found and not "given." If they

had been "given," the experiments producing them would presumably not have been necessary. Use of the right word, instead of "data" in the wrong sense, sidesteps the issue of its grammatical number.

As to that issue itself, no settlement seems likely between those who write "the data is" and even "the strata is" on the analogy of "agenda"—and, I might add, "opera"—and those who cling tenaciously to the old-fashioned view that Latin plurals should remain plural as long as possible. Argument is probably of little avail when, as here, matters of syntax merge into matters of taste, for "*de gustibus...*"

A. L. BACHARACH

26 Willow Road, London

A Blood Pump for Whole Blood Without Anticoagulants¹

WE HAVE designed and used for the past year a blood pump that will operate efficiently on whole, nonheparinized blood, without causing clotting.

Many different types of "artificial hearts" have been designed to pump blood. In general these pumps have required the use of an anticoagulant such as heparin, which is often an obvious disadvantage, particularly in surgery. The recently reported use of an "artificial heart" on a human patient (1) during cardiac surgery required heparinization. The one reported pump designed to operate on blood without addition of anticoagulants (2) required a lining made from a dissected blood vessel, preparation of which is a difficult technical procedure.

To prevent clotting in its mechanism, a blood pump should ideally have a very short circulation time, entirely smooth inner surfaces, no dead or serious eddy space, and should be free of valves. Also, the blood should be in continuous motion at rather high velocity at every point and must never touch anything other than a nonwetting surface.

The DeBaKey roller pump (3), which squeezes a length of rubber tubing by means of a roller, meets many of these criteria, but a rubber surface in contact with blood initiates clotting. Recent development of nonwetting and nonreactive surfaces has made it possible to handle whole, coagulable blood outside the body in silicone-coated or plastic containers for considerable periods of time before clotting occurs (4), but it has been difficult to give rubber a satisfactory coating that will withstand roller action. Roller action has not previously been satisfactory on a plastic tubing, because a roller pump requires a high degree of elastic rebound on the part of the tubing after passage of the roller.

We have modified the DeBaKey-type pump to obtain this elastic rebound in polyvinyl tubing by en-

¹This work was done during the tenure of a research fellowship of the American Heart Association.

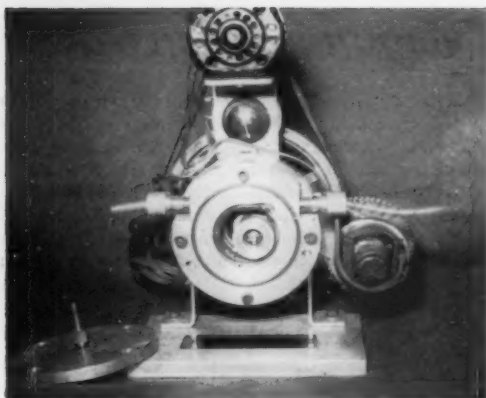


FIG. 1. Pump with casing cover removed to show eccentric rotor and loop of tubing. Motor above and generator-tachometer (used to indicate flow rate) at right.

closing the roller and a section of the tubing in a vacuum. The pump was designed to use 3/16" ID .032 wall medical-quality, clear polyvinyl tubing.² This tubing is hemo-repellent, may be sterilized by autoclaving, and does not initiate clotting. The pump is so designed that a continuous length of tubing may be passed from the blood source, through the pump, and back to discharge point. Simple seals prevent air leakage at the points where the tubing enters and leaves the pump, and at the roller drive-shaft bearing. A vacuum is maintained within the pump by suction applied to a nozzle on the airtight casing cover. Within the vacuum, a roller, mounted eccentrically, operates on a circular loop of tubing. The pump, with casing cover removed, is shown in Fig. 1.³

As used during the past year, this pump, driven by a 1/10 hp electric motor, has delivered flow rates from under 100 ml/min to 1200 ml/min. Using brass arterial cannulas coated with the nonwetting surface "Arquad 2C,"⁴ it has circulated the blood of the nonheparinized, living dog for 2½ hr without evidence of clotting. Although we have not had occasion to extend this period of operation, there seems to be no reason why it could not be used for a considerably longer time. However, tubing wear and cracking appear after 5-6 hr of continuous operation. Replacement of worn tubing is simple.

JOHN J. OSBORN

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² Supplied by Fenwal Laboratories, Inc., Framingham, Mass.
³ Engineered and manufactured to order by Halm Instrument Co., Glen Head, N. Y.

⁴ Supplied by Armour, Inc.

The Scientist and the Library Cataloger

From *Physics Today*, Vol. 4, No. 1, 28-29, January, 1951. "A Report From Washington: Library of Congress Science Division," by Dwight E. Gray:

It is inherent in the very idea of dividing things into categories that a classification system which is highly satisfactory according to one criterion is bound to be quite unsatisfactory on some other basis; that is to say, one man's orderly arrangement may be another's hodgepodge. A physicist, a chemist, and an engineer, for example, might wish respectively to classify a given group of substances on the basis of their physical properties, their chemical compositions, and their industrial applications, and the grouping of no one of the men would be wholly satisfactory to the other two. Or, a breakdown of college coeds according to intelligence quotients might be very useful to the dean but quite worthless to the man-about-campus whose major classification categories in this discipline are blondes, brunettes, and redheads. In other words, the best classification system for any given situation—whether for people, objects, or ideas—is simply the one that experience shows is the most useful.

THE title above should read perhaps "The Scientist versus the Cataloger," for the relationship between the scientist and the cataloger has often been one of estrangement and opposition and not one of understanding and cooperation. But, is it not time that the nature of this relationship be changed from mild opposition or at least lack of understanding to effective collaboration and mutual respect.

Every scientist who publishes a book, be it a monograph, textbook, or laboratory manual, must realize by now that his work will find itself among the other books in some library or libraries, where the books are arranged by subject usually according to the Dewey Decimal, the Library of Congress, or the Brussels classification system. The foreknowledge that the book will be classified by subject provides an opportunity for the scientist to suggest the specific subject under which he would like to see his work placed. That some books do not find the correct subject or the most useful classification is an evidence of utter lack of cooperation between two fields of endeavor that somehow should be joined.

A striking example is adduced for its recency without any criticism aimed at the particular author or the librarians who classified his book. The recent suggested classification of Roberts Rugh's "The Frog; its Reproduction and Development" (1951) by the Library of Congress with books on frogs and not with books on embryology as the specific subject is an evidence of the lack of cooperation between the scientist and the cataloger.

But this cooperation might have existed, if Mr. Rugh could in some manner or other have explicitly indicated that the book in question was best classified with other books on embryology.

The example is perhaps not the most apt but librarians who work with scientists know of many others.

It is suggested that cooperation between the scien-

tist and the cataloger before the cataloging process begins would constitute a guarantee against any book being placed in a secondary or outright useless category.

The cataloger would doubtless welcome the suggestions of the scientist for whereas the specialist thoroughly understands his own subject the cataloger of necessity must range through a myriad of subjects. That the cataloger cannot be a specialist in every branch of the physical and social sciences must be fairly admitted.

Could the librarians interest the scientists in thinking about the subject classification of their works?

ROGER J. POULIN

Nesmith Library, University of New Hampshire

WITH Mr. Poulin's general aim of collaboration between the scholar and the cataloger there can certainly be no objection. Indeed the Library of Congress makes a considerable effort to have special subject knowledge represented in its staff, both in the reference and cataloging activities. It collaborates with organizations of specialists in a number of activities, and suggestions for extending this collaboration are always welcome.

Implicit in part of Mr. Poulin's statement, however, is an assumption which we believe is unsound, namely, that the classification of any given book in any given classification scheme is a matter of absolute precision; that is, that there is one correct number and no other. In many cases, this assumption is warranted, but in many others it is not. The latter are those of works which deal with more than one subject; or with one subject in more than one aspect; or that treat a general subject by means of specific illustration; and so on. In all of these situations we believe it is not possible to maintain that there is one best number, even from an abstract point of view, and clearly not from the point of view of the best placement of a particular book in a particular collection.

On the basis of this general proposition, we believe that there is a case for classifying the *Rugh* mono-

graph on the frog either with works on the *Salientia* (QL668.E2) or with the embryology of vertebrates (QL959). It happens that the Library does not want to press the case in this instance, for our review of the book shows that QL669 (*Batrachia—Anatomy & Physiology*) is clearly incorrect and that QL668.E2 (*Frogs and Toads*) would not have been in accordance with our prior decisions in this field of knowledge. Mr. Poulin has called our attention to an error and we are accordingly reclassifying the book in QL959 (*Embryology*).

From a general point of view, however, it seems to us that there is some validity to another possible classification decision, namely, that all works on frogs go with frogs and that the section on embryology be reserved for works dealing with the embryology of more than one genus. In other words, if there is a library whose users would be better served by such a grouping, we would not hold it "wrong" classification. In the Library of Congress there are no doubt a number of such decisions that have proved to be unwise in the light of later developments. Some of these can not be changed for a practical reason: the cost is out of proportion to the benefit when weighed against other work load requirements. There are other decisions which we might have to insist were better in relation to our particular needs, even against expert opinion—with which we might well agree—that different decisions were better abstractly or in general.

It follows from these observations that, while the Library would welcome the opinion of writers and scholars on the classification of particular books (in the present case, Dr. *Rugh's* statement is unexceptionable from this point of view) it would want writers to understand that its departures from their recommendations should be attributed to differences of need and of judgment and not to wilfulness or—except very rarely we hope—ignorance.

RICHARD S. ANGELL

*Subject Cataloging Division
Library of Congress*

Book Reviews

Dental Anatomy: Including Anatomy of the Head and Neck. 3rd ed. Moses Diamond. New York: Macmillan, 1952. 471 pp. + plates. \$15.00.

The author of this book has divided his subject into 20 chapters covering the complete anatomy of the head and neck regions in relation to the dental apparatus. His principal object is a detailed description of the morphology of each of the individual teeth to facilitate the art of dental reproduction.

For the purpose of describing each of the individual teeth, Dr. Diamond has established a basic tooth form that he has chosen to call the "symmetrical tooth form."

This he has done by the elimination of variations and anomalies which individualize a particular crown. The description of the symmetrical crown form is subsequently built up from a description of the segmental portions and their arrangements as they comprise the whole. This basic form has been established for each of the 32 adult teeth.

The author's descriptions are clear and concise, although he has deviated slightly in some instances from the current nomenclature. In addition to the detailed description of each tooth, he has presented a systematic technique for reproducing the symmetrical crown

form. The author has thus taken a natural science and made it an applied science.

A separate chapter is devoted to variations and anomalies as related to the mechanism of their origin. This arrangement aids the dental practitioner in adding variations to the symmetrical crown form for individual dental restorations.

The remaining chapters on muscles of mastication, blood, and nerve supply, maxilla and mandible, and soft tissues of the mouth give a more or less comprehensive study of the dental apparatus in one volume. For students of tooth morphology, however, the treatment is repetitious of the study of these structures in gross anatomy.

EDMOND G. VANDEN BOSCHE

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A History of Psychology in Autobiography, Vol. IV. E. G. Boring, H. S. Langfeld, H. Werner, and R. M. Yerkes, Eds. Worcester, Mass.: Clark Univ. Press, 1952. 356 pp. \$7.50.

Volumes I, II, and III of this series were published between 1930 and 1936 by Clark University Press under the editorship of Carl Murchison. After Murchison left Clark, the series lapsed for many years. With the appearance of the present volume, this series has now been reinstated.

The editors of this volume constituted a committee appointed by the American Psychological Association for the specific purpose of producing this volume. The committee chose the men invited to contribute and obtained and edited the autobiographies. Lives of the following men were included: Walter Van Dyke Bingham, Edwin Garrigues Boring, Cyril Burt, Richard M. Elliott, Agostino Gemelli, Arnold Gesell, Clark L. Hull, Walter S. Hunter, David Katz, Albert Michotte, Jean Piaget, Henri Pieron, Godfrey Thomson, L. L. Thurstone, and Edward Chace Tolman.

The editors indicate that they chose men over 60 years of age, "who might be expected to have acquired a sufficient past to make an account of it worthwhile." The standards for inclusion, while otherwise not stated, were obviously severe. An examination of the *Directory* of the APA shows that in 1951 there were 420 persons over 60 years of age in this organization. For this volume, eight Americans were chosen, or only 2% of the total. Of these eight, all have been starred in *American Men of Science*, and five have been presidents of the American Psychological Association. Similar data are not available for the psychologists in this volume who represent the countries other than America, but it seems likely that these men are comparable in level of selection to the American representatives. It is not surprising that the non-Americans number only seven because psychology has been peculiarly American since Hitler and other dictators silenced, or forced to America, many psychologists from other nations. Doubtless more than half of the world's emi-

nent psychologists now live in America, although many of them came from elsewhere.

Although they are fascinating reading, the individual autobiographies cannot be reviewed here. It should be noted, however, that they differ tremendously among themselves. This is due partly to the diversity among psychologists, in regard to personality, fields of interest, and professional history. It is due also, it would seem, to the fact that the editors did not coerce the authors into a common pattern of content or style. An assignment of this kind would seem to constitute the best projective technique for the study of the individual. Although the biographies are primarily phrased as "intellectual histories," references to personal, social, and emotional development are by no means absent and personality shows through the semi-transparent scientific record. These life histories will be of value to the students of history, of science, and of personality, as well as to those interested in the history of psychology.

A book of this kind is particularly valuable because, in the past, few scientists have written autobiographies. This probably stems from the fact that the likelihood of commercial publication of a full-length autobiography by a scientist is small. The *History of Psychology in Autobiography*, each volume depicting more than a dozen important lives, demonstrates a feasible way of publishing the autobiographies of men of science. These are the persons who are increasingly placing within the power of man the ability to control nature and man. Such men need to be understood, and autobiography provides one path to that understanding. The Clark University Press is to be congratulated upon this series in psychology. It would be fortunate if a comparable series were extant in each of several other fields of learning.

WAYNE DENNIS

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Scientific Book Register

The Chemistry of Synthetic Dyes, Vol. II. K. Venkataraman, New York: Academic Press, 1952. 738 pp. Illus.
British Pharmacopoeia 1953. London: Pharmaceutical Press, 1953. (For the General Medical Council). 894 pp. 50s.

Nuclear Stability Rules. Cambridge Monographs on Physics. N. Feather. New York: Cambridge Univ. Press, 1952. 162 pp. Illus. \$4.00.

Glycerol. American Chemical Society Monograph Series. Carl S. Miner and N. N. Dalton, Eds. New York: Reinhold, 1953. 460 pp. Illus. \$12.00.

Small Particle Statistics. An account of statistical methods for the investigation of finely divided materials. G. Herdan; with a guide to the experimental design of particle size determinations by M. L. Smith. Amsterdam-Houston: Elsevier, 1953. 520 pp. Illus. \$12.00.

Erratum. The series of *Gmelins Handbuch der anorganischen Chemie* reviewed in the April 17, 1953 issue of *SCIENCE*, pages 424-25, is distributed in the U.S. by Stecher-Hafner, Inc., 31 E. 10th St., New York, and by Walter J. Johnson, Inc., 126 E. 23rd St., New York.

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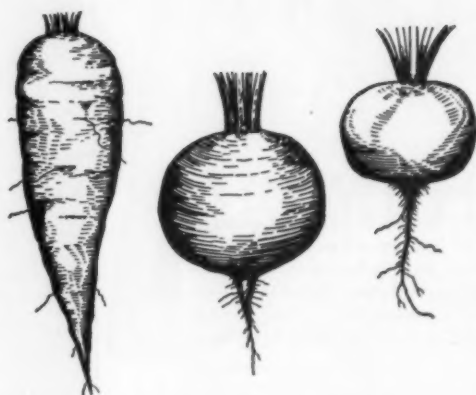
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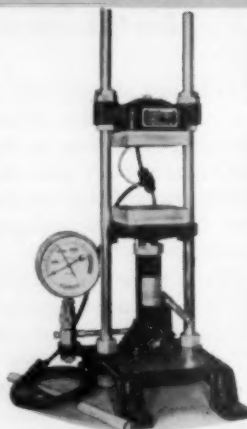


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- May 25-30. International Hospital Congress. London.
- May 25-30. International Seed Testing Association (10th Congress). Dublin.
- May 25-31. World Congress of Fertility and Sterility. Henry Hudson Hotel, New York.
- May 28-29. American Rheumatism Association. Waldorf-Astoria, New York.
- May 29-31. Field Conference of Pennsylvania Geologists (Annual). Lafayette College, Easton.
- May 30-31. Society for Investigative Dermatology (Annual). Hotel Belmont Plaza, New York.
- June 1-5. American Medical Association (Annual). New York.
- June 4-6. Canadian Society of Microbiologists. Ontario Agricultural College, Guelph.
- June 5-6. International Congress of Audiology. Leiden.
- June 6-7. International Congress of Bronchology. Utrecht.
- June 7-9. American College of Cardiology (Annual). Hotel Statler, Washington, D. C.
- June 8-13. International Congress of Otorhinolaryngology (5th). Amsterdam.
- June 9-13. American Dermatological Association. Lake Placid Club, Essex County, N. Y.
- June 10-12. Research and Development Associates (Annual). Mayflower Hotel, Washington, D. C.
- June 10-20. General Chemistry and Analytical Chemistry Workshop. Pennsylvania State College.
- June 12-13. Wilson Ornithological Club. Sheboygan, Mich.
- June 12-14. American Medical Technologists (Annual). Hotel Hollenden, Cleveland, Ohio.
- June 14-18. American Society of Medical Technologists (Annual). Brown Hotel, Louisville, Ky.
- June 14-18. Canadian Gas Association. Windsor Hotel, Montreal.
- June 15-17. American Society of Agricultural Engineers (Annual). William Penn Hotel, Pittsburgh.
- June 15-17. American Neurological Association (Annual). Hotel Claridge, Atlantic City, N. J.
- June 15-18. American Society of Mammalogists. American Museum of Natural History, New York.
- June 15-18. American Chemical Society Organic Chemistry Symposium. University of Michigan, Ann Arbor.
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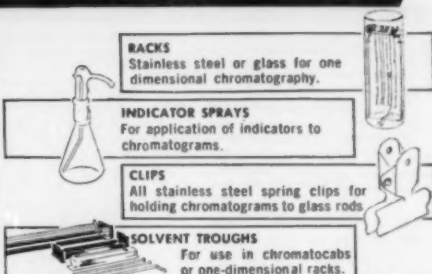
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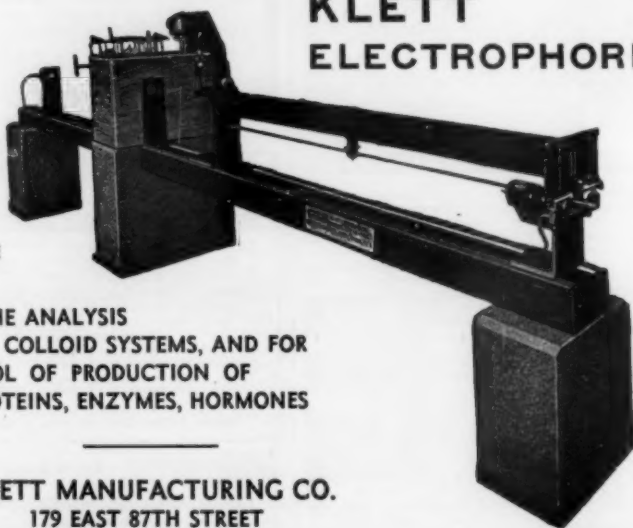
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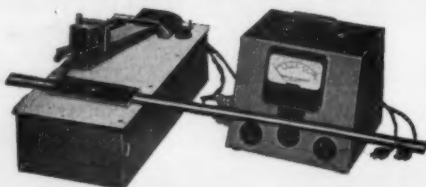
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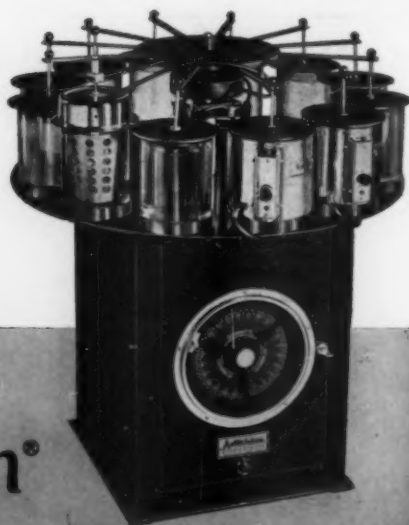
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